A PRELIMINARY STUDY OF COMPLEMENT SYSTEM IN CHILDREN WITH PROTEIN-CALORIE MALNUTRITION

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C E R T I F I C A T E

This is to certify that the work entitled "A PRELIMINARY STUDY OF COMPLEMENT SYSTEM IN CHILDREN WITH PROTEIN - CALORIE MALNUTRITION" has been carried out by Anil Kumar, under my direct supervision and guidance in the Department of Paediatrics, M. L. B. Medical College, Jhansi.

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Akumar

(ANIL KUMAR)

' Nation marches on the tiny feet of little individuals, and hence no nation can afford to ignore its children'

- Jawaharlal Nehru

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INTRODUCTION

A cornerstone of the present century lies in the enormous advances made by unpteen workers in the field of immunology. Although the immunological profile of a host of Paediatric diseases has been studied so far, the immunologic study of 'Protein-Calorie Malnutrition' holds a special significance owing to its devastating effects on the growth and development of the child.

Nutrition is an essential part of the mosaic of factors that determines the natural history and biological gradient of the disease, especially in the developing world. In no other area this statement is more dramatically illustrated than in the interaction of malnutrition and immunity.

In India, children below 14 years of age constitute 42.8% while those below 5 years constitute 17% of the total population. Pre-school children not only form the bulk of child population but this period of childhood, especially the second year of life, is notoriously fraught with risk. The young child is "transitional" as regards diet, immunity to infections and psychological dependence. It is at this stage of rapid growth, exploration and interaction with the environment that a child is prone to encounter accidents, develop malnutrition and infections and suffer from behaviour problems.

Protein-calorie malnutrition (PCM) is the commonest child health and social problem affecting vast areas of the world. Obviously the condition is more prevalent and endemic in developing and under developed countries. PCM covers the whole range of mild to severe, classifiable and unclassifiable manifestations of malnutrition, including the two main clinical syndromes of kwashiorkor and nutritional marasmus. One important consequence of PCM is the retardation of child's growth and development. Mortality in children, especially among pre-school children, is closely related to nutritional status.

Nutrition, immunity and infection are known to be closely linked. Inadequate nutrition can alter the immunocompetence and thus increase susceptibility to infection. Infection, inturn, can adversely affect nutritional status.

Children with PCM are known to be unusually susceptible to infections, which are often more severe and slower to resolve, than is the case with those having normal nutrition. This has been attributed mainly to their altered immunocompetence. PCM causes depression of several host defense mechanisms including phagocytic and killing functions of leucocytes, cell mediated immune response, inflammatory response and antibody production.

The role of the complement in host defence mechanisms is well established. It is one of the principal mediators of the immune response and is capable of causing lysis of cells, bacteria and viruses. The complement system comprises a series of the proteins which require sequential activation for the biological action. Genetic deficiencies of the complement proteins are known to be associated with recurrent infections.

Recent reports have suggested that PCM adversely affects complement system, which may account in part for the increased susceptibility of malnourished children to infections.

It is in the light of these observations that the present venture though a humble one is directed at studying immunological profile of malnourished children. The study aims at the following :-

1- To evaluate the complement system in pre-school children having protein-calorie malnutrition, so as to assess their immunological status in relation to the severity of malnutrition.

2- To ascertain possible inter-relationship, if any, between the clinical progress of case and subsequent change in complement activity.

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REVIEW OF LITERATURE

PROTEIN-CALORIE MALNUTRITION :

Historically, marasmus (Greek marasmos, wasting) was recognised for hundreds of years as being, with gastro-enteritis, a major contributor to high infant mortality. In the early part of this century reports from central Europe of so called 'Starch dystrophy' attracted little attention. The classic description by Williams (1933) of a disease attributable to protein deficiency, which she named 'Kwashiorkor' (taken from the 'Ga' language of Ghana), recognised that this was the disease, the first child got when the second was on the way. It was characterised by skin and hair changes, oedema, moonface, fatty liver, hypoalbuminaemia and psychomotor changes. Clinical descriptions of a disease, obviously similar, appeared from many other countries although in the West Indies for example, the dermatosis was uncommon, oedema prominent and the term 'Sugar baby' was subsequently used by Waterlow (1948) and Jelliffe et al (1954).

Jelliffe (1959) coined the term 'Protein-Calorie Malnutrition (PCM)' of early childhood to include the mild and moderate degrees and all the clinical types of the severe degree of malnutrition. Using a variety of biochemical tests Nolaren et al (1967) were able to show that the severe degree of PCM in its various clinical forms of marasmus, marasmic-kwashiorkor and kwashiorkor formed a spectrum of both clinical signs and biochemical changes; both being most marked in full blown kwashiorkor and least evident in pure marasmus.

There was a short lived effort through World Health Organization (WHO) to introduce the term 'Protein-calorie deficiency diseases' but this was abandoned by the expert group meeting in 1970 in favour of PCM. Since then, the impact of proposals to replace the term calorie by Joule as a unit of energy measurement has led to a general use of the word 'Protein-Energy Malnutrition' rather than Protein-Calorie Malnutrition. At present there is an increasing recognition of the fact that the major problem all over the world is deficiency of food intake in general (and therefore of energy) rather than of protein in particular. Again, to emphasize that this is but part of the overall energy crisis of mankind the term energy-protein malnutrition or EPM has been used by workers to give the needed stress to energy deficit (McLaren, 1976).

Magnitude of the Problem :

PCM is one of the world's major public health problem especially in the underdeveloped and developing countries. Rao et al (1969), in a study of pre-school children of rural communities, found that the percentage prevalence of frank cases of kwashiorkor and marasmus were 0.6 and 1.0 respectively. In a survey of rural pre-school children Ghai et al (1970) reported that about 18 percent of all cases were undernourished. Out of these 1.7 percent had nutritional marasmus and 0.9 percent suffered from kwashiorkor.

Point prevalence figures have been collected for a number of years by WHO. Bengoa (1974) reported data from 77 nutrition surveys in 46 developing countries, totalling near 2 lakhs children mostly under 5 years of age and suggested that about 100 million children throughout the world were suffering from moderate or severe PCM at any one time. Bistrian et al (1974) have brought attention to the large number of patients with secondary malnutrition who were present in the wards of United States hospitals.

Gopalan (1974) computed that nearly 65 percent of toddlers in poor communities in India suffered from moderate malnutrition and 10 percent from severe malnutrition. Ghai (1978) analysed severe cases of PCM in hospitals and reported 6.6 percent deaths in cases suffering from marasmus and 33.3 percent in kwashiorkor and marasmic-kwashiorkor.

Ghai (1977) showed that malnutrition was a major contributory cause of mortality in about 40 percent of childhood deaths, even though it was often not listed as a primary cause of death in most of studies. Rao (1978) reported that marasmus and kwashiorkor, were seen only in 1-2 percent of the pre-school child population. As many as 60-70 percent of the children, on the other hand, suffered from mild and moderate degree of PCM.

Recently Ghosh (1981) has shown that in India, there were about 100 million pre-school children out of which 3 to 4 million suffered from severe types of malnutrition, and probably 1 million of them died.

Classification (Grading) :

Grading of PCM is necessary for formulating therapy in individual patients and defining priorities for combating malnutrition. Three main direct methods of assessing PCM in the community have been used - clinical, anthropometry and biochemical.

Gomez et al (1955) is credited with the first ever classification of malnutrition, using the actual weight expressed as a percentage of standard weight (Boston 50th percentile) for age. The presence or absence of clinical characteristics such as oedema was not taken into account by the authors.

GOMEZ CLASSIFICATION

Grade of malnutrition	Weight
Normal	⅂ 90% of expected weight for age.
Mild (1st degree)	89 - 75 %
Moderate (2nd degree)	74 - 60 %
Severe (3rd degree)	∠ 60%

In a later modification, Jelliffe (1966) included all cases with nutritional oedema, irrespective of weight, in 3rd degree.

Although weighing scales may not always be available or maintained or used correctly and age is often not known accurately, yet this method is in common use. Its main drawbacks are, that it assumes all children of certain age to have the same weight, irrespective of their size as measured by height for example. It also includes such children who are underweight as a result of malnutrition in the past.

McLaren et al (1967) introduced a simple scoring system for classifying the severe forms only (satisfying Gomez criteria of weight $\leq$ 75%), based on all three methods of assessment viz. clinical, anthropometric and biochemical.

MCLAREN CLASSIFICATION

Signs present			Points
Oedema	...	...	3
Dermatosis	...	...	2
Oedema plus dermatosis		...	6
Hair change	...	...	1
Hepatomegaly	...	...	1
* Serum albumin (Total serum proteins)			
(g/100 ml)	(g/100 ml)		
$\leq$ 1.00	($\leq$ 3.25)	...	7
1.00 - 1.49	(3.25 - 3.99)		6
1.50 - 1.99	(4.00 - 4.74)		5
2.00 - 2.49	(4.75 - 5.49)		4
2.50 - 2.99	(5.50 - 6.24)		3
3.00 - 3.49	(6.25 - 6.99)		2
3.50 - 3.99	(7.00 - 7.74)		1
$\geq$ 4.00	($\geq$ 7.75)		0

Score = Sum of points ; 0-3 = marasmus;
4 - 8 = marasmic-kwashiorkor ; 9 - 15 = kwashiorkor.

* Either serum albumin or total serum protein were used for assessment.

This system has been used by a number of centres and is the only method available at present for a fairly precise and objective classification of the type of patients studied in hospitals. The problem of expressing chronicity and stage of disease however remains unsolved.

Some classifications have been designed to use measurements requiring only simple apparatus, avoiding the necessity for calculations and also the need to know the age of the child. These could be thus applicable under routine field conditions by unskilled personnel. Among these, Quacstick (Arnhold, 1969) method uses the height and mid-arm circumference. Based on this classification children were divided into two broad categories, 'malnourished' and 'normal'.

The ratio of mid-arm circumference/head circumference was shown to be independent of age at least from 3 to 48 months and was similar in either sex (Kannusti and McLaren, 1970). Based on this ratio, the following classification has been proposed to detect cases of malnutrition.

Ratio	Classification
0.310	Nutritionally healthy.
0.310 - 0.280	Mild PCM
0.279 - 0.250	Moderate PCM
0.250	Severe PCM

However, it needs to be emphasised that the method is rough, should not be used for individual children and is meant to screen large numbers.

The classification that appeared in the 8th report of FAO/WHO Expert Committee (1971) is one that was originally prepared by the Wellcome Trust and is sometimes referred to as the 'Wellcome' classification.

WELLCOME CLASSIFICATION

	Body weight as % of standard*	Oedema	Deficit in weight for height**
Underweight child	80-60	0	Minimal
Nutritional dwarfing	$\angle$60	0	Minimal
Marasmus	$\angle$60	0	++
Kwashiorkor	80-60	+	++
Marasmic-Kwashiorkor	$\angle$60	+	++

* Standard taken as 50th percentile of the Harvard values.

** Weight for height = $\frac{\text{Weight of patient}}{\text{Weight of normal subject of same height}} \times 100$

'Wellcome' classification was probably the first in which an attempt was made to use weight/height as well as weight/age ratios and included a separate category of 'nutritional dwarfs'. However, it has some notable deficiencies. It confuses between the type and severity

of malnutrition. Diagnosis of marasmus, marasmic-kwashiorkor, and kwashiorkor refer to differences in the type of malnutrition and all are of similar severity. Thus in this system kwashiorkor appears to be less severe than the other two types as the body weight is 60-80 % of standard and not below 60%. Gradation of deficit in weight for height by such terms used as 'Minimal' and '++' can not be quantitated.

Nutrition Sub-Committee of the Indian Academy of Pediatrics (1972) classified PCM into 4 grades using 50th percentile of Harvard growth standard as a reference point.

CLASSIFICATION OF INDIAN ACADEMY OF PEDIATRICS

Grade of malnutrition	Weight expressed as percentage of reference standards.
I	71 - 80 %
II	61 - 70 %
III	51 - 60 %
IV	$\angle$ 50 %

Grade I and II are underweight and grade III and IV correspond to marasmus. When nutritional oedema is present, letter 'K' is suffixed to the grade denoting malnutrition, eg, 1K; 2K etc. 1 K and 2 K will mean kwashiorkor and grade 3 K and 4 K will correspond to marasmic-kwashiorkor.

Waterlow and Rutishauser (1974) published a classification based on weight and height, thus taking into account the effect of past as well as present malnutrition. The 'present' malnutrition was called 'Wasting' as measured by loss of weight in relation to height, and 'past' malnutrition called 'Stunting' was seen as low height for age ratio.

WATERLOW AND RUTISHAUSER CLASSIFICATION

Grade	Stunting (height for age)	Wasting (Weight for height)
0	7 95%	7 90%
1	95-90%	90-80%
2	89-85%	80-70%
3	L 85 %	L70 %

Waterlow maintained that weight/height was independent of age, basing his argument on two sets of data which were collected on children age between one and four years.

MALNUTRITION, INFECTION AND IMMUNITY :

Scrimshaw et al (1968) reported that the nutritional status was a critical determinant of susceptibility to infection. He supported his clinical impression by epidemiological data and experimental studies in laboratory animals.

Philips et al (1968) also found that children with PCM were unusually susceptible to severe infections and took longer time to combat such infections.

Ramalingaswami and Ramalingaswami (1973) observed that malnutrition and infection, singly and in combination, contributed significantly to morbidity and mortality of infants and children in the developing countries.

In another comprehensive study, Reddy et al (1978) concluded that nutrition, immunity and infection were closely linked. They showed that inadequate nutrition could alter the immunocompetence, thus increasing the susceptibility to infection, and infection in turn, adversely affected nutritional status.

Defence Mechanisms in PCM :

In defence against bacteria, viruses and other pathogens, several facets of immunocompetence come into play. Phagocytic activity and bactericidal competence of leucocytes constitute the first order of defence. In addition, two other types of immune mechanism, which operate against infection, are the humoral and cell mediated immunity. Also, there are other nonspecific defence factors such as lysozyme, complement and opsonins which play an important role in determining resistance to infection. Alternations in one or more of these mechanisms may be expected to increase susceptibility to infections.

PCM causes depression of several defence mechanisms.

Smythe et al (1971) demonstrated profound depletion of the thymolymphatic system and severe depression of cell mediated immunity in malnutrition.

Chandra (1972) noticed that antibody response to tetanus toxoid was adequate, but response to S. typhi vaccine was significantly reduced in malnourished children. He also reported depressed cell mediated immune response in PCM.

Selvaraj and Bhat (1972) and Seth et al (1972) showed that phagocytic and killing functions of leucocytes were decreased in children with PCM.

Edelman et al (1973) observed depressed inflammatory response and cell mediated immune response in PCM.

Reddy et al (1977) showed that both the cell mediated immune response and antibody response to bacterial antigens were impaired in children with severe PCM. However, the immunological responses were not altered in those with mild to moderate PCM as observed by authors.

Kumar et al (1978) observed depressed cell mediated immunity in children with PCM.

Puri et al (1980) reported that various parameters of cellular immune response were significantly depressed in severe PCM. However, the authors also

observed that humoral immunity was not altered in PCM except in the presence of infection, when there was some increase in IgG levels.

COMPLEMENT SYSTEM :

Complement is a system of factors occuring in normal serum which are activated characteristically by antigen-antibody reaction and subsequently mediate a number of biologically significant consequences. It is now apparent that complement acts as the principal mediator of the inflammatory response and plays an essential role in host defences against infection.

According to McConnell and Lachmann (1976) and Peppys (1976), role of complement has advanced in recent years from being a collection of obstruse biochemical phenomenon to a system which has fundamental importance in immunogenetics and immunopathology.

History :

Pfeiffer (1894) demonstrated that the immune system of guinea pigs acquired the capacity to dissolve cholera bacteria (Pfeiffer's phenomenon).

Bordet (1896) repeated the experiment and found that Pfeiffer's phenomenon required two components of serum : a heat stable component (stable at 56°C for 30 minutes) that was present only in immune serum and a heat labile component present in immune as well as nonimmune sera. Bordet described the same phenomenon

in the serum of animals immunized with red blood cells of different species and called heat labile factor 'Alexin'. The term alexin was later replaced by the new term 'Complement' proposed by Ehrlich and Morgenroth (1899). These authors concluded that serum contained two substances : the interbody having two haptophore groups (analogous to immune body) and an addiment, which they named complement because it completed the antibody's immune response after it reacted with antigen.

By the 1920s there were 4, by the 1960s, 9 components were known (one of which had 3 subcomponents). Austen et al (1960) labelled the original system of 11 interdependent factors as the classical pathway of complement.

Gotze et al (1971) and Gewurz (1972) described a second major pathway of activation of complement, the alternative or properdin pathway. Authors also reported that this system consisted of at least 4 factors.

Basic Precepts :

Johnston and Stroud (1977) described the basic precepts of complement system :

1. Complement is a system of interacting proteins. The biologic functions of the system depend upon the interaction of individual components.
2. The components interact in an orderly, sequential fashion. This has been referred to as 'Cascade', in that activation of each component (except the first) depends upon activation of the prior component or components in the sequence.

3. Interaction occurs along two pathways :

The Classical Pathway- in which the components interact in the following order : Antigen-antibody C142356789, and the more recently discovered alternative or Properdin Pathway. In this alternative pathway the chain of reaction is : Activator (antibody) — Properdin system — C356789. Whether an antibody is required and what is the exact sequence of interaction of components in the alternative pathway is still not clearly understood.

4. The interaction of early acting components (C1423) is enzymatic in nature, so that "activation" refers to transformation of the components into an active enzyme. In contrast, the interaction between C5b, C6, C7, C8 and C9 is non-enzymatic through non-covalent, probably hydrophobic, bonds. In the case of C1, activation is a result of its interaction with antibody. Activation of C4, C2, C3, C5, as well as of factor B of the alternative pathway, is secondary to cleavage by a preceding component or components. This activation of early components generates an enzyme which fixes to the antigen-antibody complement complex and catalyses a reaction on the next component, whereas later acting components (C6 to C9) adsorb on to the complex or the underlying cell by an interaction which depends on a change in their configuration.

Sequence of Activation :

Johnston and Stroud (1977) summarised the sequence in which the components of the classical pathway and alternative pathway interacted. The interdigitation between classical and alternative pathways and the classical and functional by products of these reactions were also described (Fig.: 1, 2).

THE BIOLOGICAL ROLE OF THE COMPLEMENT SYSTEM :

Fust (1978/1979) inferred that the complement system played an essential role in a number of physiological processes participating in the defence mechanism of the organism and were mostly favourable. However, he emphasised that like other plasma enzyme systems, complement played a dual role. He expressed the opinion that events occuring during complement activation and the substances liberated during such activation could induce pathological processes. To substantiate his opinion, author quoted the example of complement playing an essential defensive role in the elimination of immune complexes (ICs) while it also caused tissue destruction.

Participation in Host Defense :

Dias da Silva et al (1967) and Shin et al (1968) expressed that complement was a dominant force in mediating inflammation, phagocytosis and cytolysis. They showed that when the functional unit was activated, both C3a and C5a allowed histamine release from mast cells

The Complement System

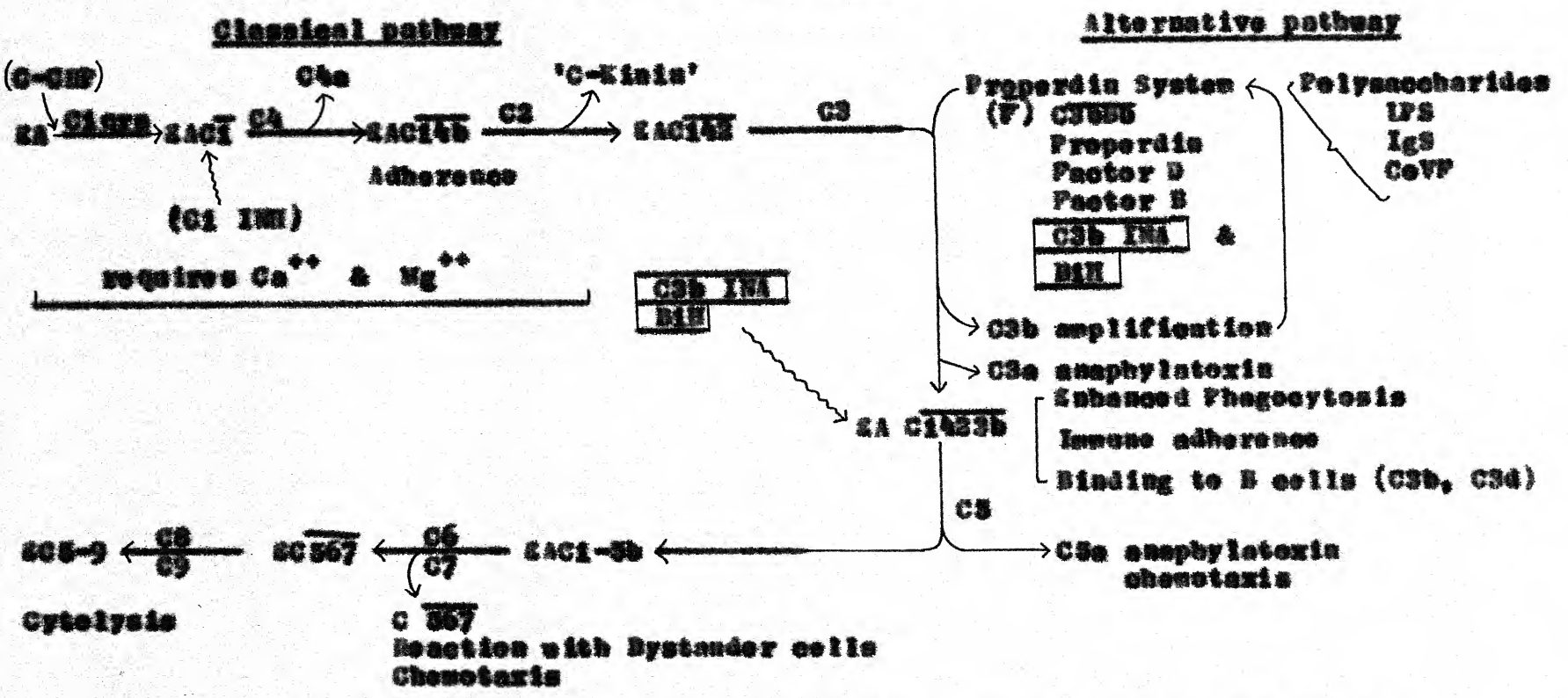

Fig. 1 : Sequence of Activation of the complement components of the classical pathway and interaction with the properdin system. E = Erythrocyte (could stand for any antigen 'Ag' eg. bacterium, virus, tumour cells); A = Antibody (Ab) (of IgG or IgM classes only); C-CHP = C - Carbohydrate - C-reactive protein; Ci INH = Ci inhibitor; C3b INA = C3b inactivator; IgS = Immunoglobuline; LPS = Lipopolysaccharides.

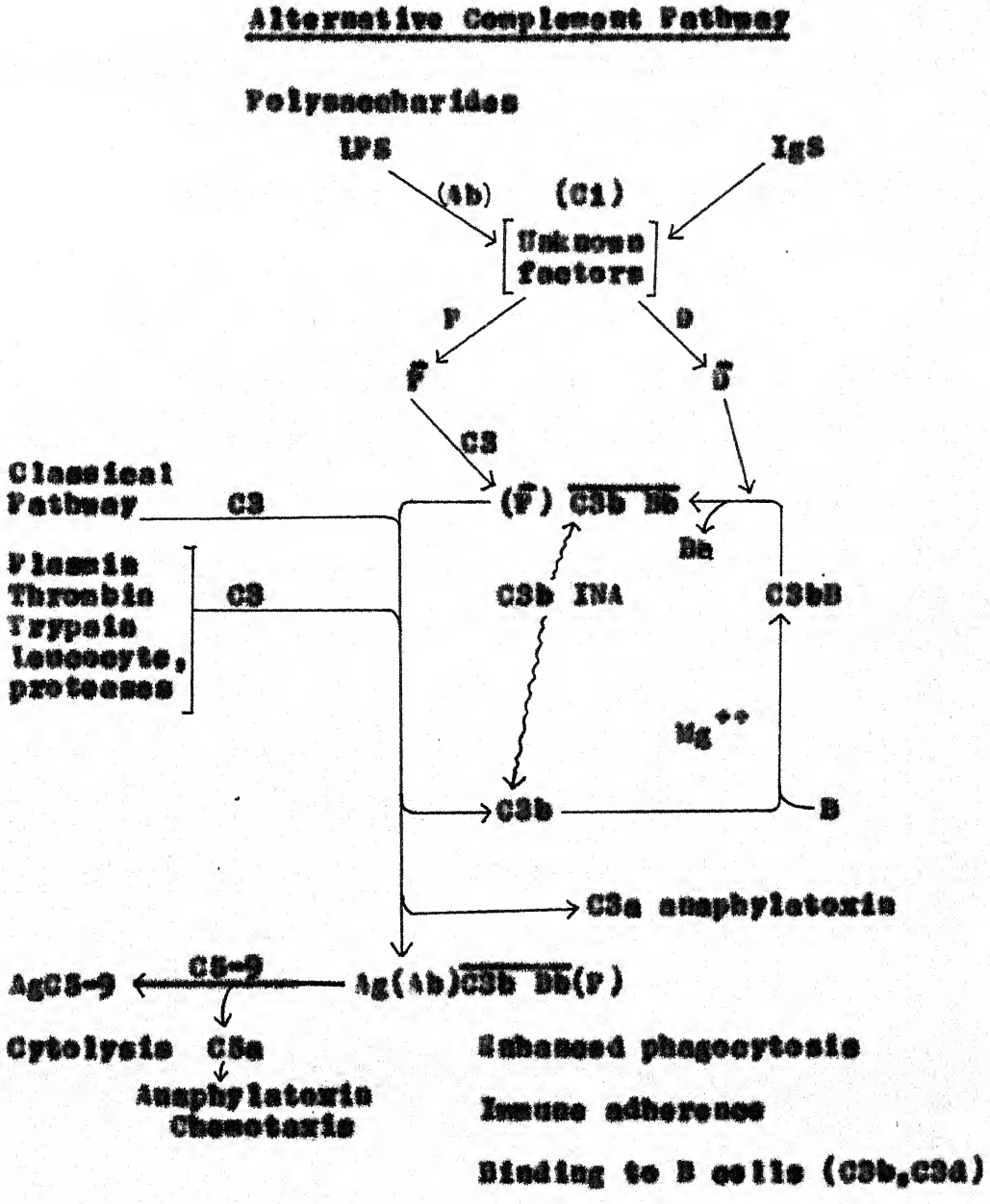

Fig. 2 : Interaction of components of the alternative (properdin) complement pathway; Ab = Antibody; Ag = Antigen.

and basophils. Besides, there were muscle contractions, an increase in capillary permeability and leucotaxis of neutrophils, eosinophils and mononuclear cells.

Mother (1972) reported a small molecular weight cleavage fragment (C3a?) from C3 which was responsible for release of neutrophils from bone marrow.

Complement forms a vital link in host resistance to infection. During infection with bacteria, parasites and yeasts, the processes of cytolysis and phagocytosis occur. Allison (1974) observed that with viral infections, cytolysis and phagocytosis did occur but in addition, complement also participated in the process of neutralization. Author described that this process either prevented the virion from entering into the target cell or that it interfered with the replication of virion inside the cell.

Strauss et al (1975) stressed that C3 activation might be important for initiating oxidative metabolism of the polymorphonuclear leucocytes and the release of lysosomal enzymes.

Koopman et al (1976) reported that by interaction with B type lymphocytes, C3b mediated the release of chemotactic factors from macrophages, B cell proliferation and formation of antibody.

Miller et al (1976) observed that neutralization of virus required either the deposition of C1, C4 and C2 or the fixation of C3 on to the virus. Depending on the

type of virus, activation and deposition of C3 could occur by the classical or alternative pathway. Antibody was not necessarily required in complement activation since some viruses could directly activate C1 by contact, as observed by the authors.

Spitzer (1977a) reported that besides providing for various elements of inflammation, complement was also involved in a direct attack on pathogenic agents by cytolysis, the entire functional unit being necessary for this purpose whether through classical or alternative pathway. Author also showed that by the deposition of C3b on the surface of the offending organism, complement system promoted phagocytosis by providing a contact point between the organism and phagocyte thus allowing internalization. This fact was further substantiated by Johnston and Stroud (1977). Johnston and Stroud (1977) summarized specific activities of the complement system in host defence against infection as follows :

ACTIVITIES OF COMPLEMENT IN HOST DEFENCE AGAINST INFECTION

Components or Fragments	Functional activity
C1q, C1r2s	Virus neutralization.
C3a, C5a	"Anaphylatoxin" (Capillary dilatation)
C3 & C5 fragments,	Chemotaxis of PMNs,
C567	Monocytes, eosinophils
C3b	Opsonization
C3b, C3d	Enhanced induction of antibody formation.
C3b	Stimulation of B-cell lymphokine production.
C3 Cleavage product	Induction of granulocytosis
C5	Opsonization of fungi
C1-6 (?additional components).	Endotoxin inactivation
C1-9	Lysis of viruses, virus infected cells, tumour cells, mycoplasma, protozoa, spirochetes and bacteria.

Fust (1978/1979) suggested that alternative pathway represented the first defence line against bacterial infections; it was capable of reacting with bacteria, opsonising them and supporting their elimination before the specific antibody response would start.

The Role of the Complement System in the Elimination of Immune Complexes (ICs) :

There are different complement mediated processes which cooperate in IC elimination.

According to Gigli et al (1968) and Ruddy et al (1972), the ICs, bearing C3b on surface, were capable of binding to the C3b receptors of polymorphonuclear leucocytes and also to the cells of the mononuclear phagocyte system. Thus ICs were finally phagocytosed.

Miller et al (1975) reported that C3b in the immune complex could change the conformation of the complex itself. As a result, large complexes were split into the smaller ones. These smaller complexes were unable to deposit in the tissues and get ultimately detoxified.

METHODS OF EVALUATION OF COMPLEMENT SYSTEM :

1. Functional Assessment :

Functional assessment of the activity of the complement system is made by measuring the lysis of antibody coated sheep erythrocytes (for total haemolytic complement) or unsensitized rabbit erythrocytes (for alternative pathway activity) by normal human serum.

i) Total Haemolytic Complement (CH_{50}):

The technique of determination of total haemolytic complement (CH_{50}) was originated by Mayer (1961). He observed that testing for total haemolytic complement served as a useful screening procedure for most of the

diseases of the complement system. This assay depended upon the ability of all 9 classical pathway components to interact and lyse antibody coated erythrocytes. Author concluded that the dilution of serum which lysed 50 percent of the cells, determined the end point and the reciprocal of that dilution was the CH_{50} or "complement haemolysis of 50% of cells."

Spitzer (1977b) reported that some of the components might be reduced significantly in amount without causing a noticeable deviation in the CH_{50}. Thus with C3, for example, it took nearly a 50% reduction to decrease the CH_{50} since C3 was normally present in large quantities in serum. In view of this major disadvantage of this screening test, author inferred that one could not place too much emphasis on this single assay.

Interestingly, Johnston (1979) drew attention and reported that in the congenital deficiencies of one or more classical pathway components, the CH_{50} value would be zero or almost so; values in acquired deficiencies would vary with the severity of the underlying disorder. Author also strongly emphasized that this procedure should be available as a screening test to every physician.

2) <u>Alternative Pathway Activity</u> :

Platts-Mills and Ishizaka (1974) observed that unsensitized rabbit erythrocyte (RRBC) activated the

alternative pathway of complement in normal human serum. Hence authors used the lysis of RRBC to assess the functional activity of the alternative pathway components including (C3-C9) and it was expressed as AP_{50}.

3) Functional or Immunohaemolytic Assay for the Different Components of Classical and Alternative Pathway :

Fust (1978/1979) described the use of functional (immunohaemolytic) assay for the assessment of different components of classical and alternative pathway. In the procedure, great excesses of the preceding components were added to sensitized sheep erythrocytes. Then serial dilutions of the serum (under test) were added to the system to serve as the only source of complement component to be tested. Finally the latter components were added. Author showed that the concentration of tested component could be calculated from the percentage haemolysis of RBCs and the corresponding serum dilution. Titre of the component was expressed in CH_{50}/ml units (CH_{50} being the complement quantity causing lysis of 50% of the erythrocytes).

II- Immunochemical Assessment :

The immunochemical technique is an important measure of quantitative assessment of various individual complement components in the serum.

The double diffusion technique as derived by Ouchterlony (1948) is a qualitative technique, used to detect the presence of antigen or antibody in a test solution and to show antigenic cross reactivity. The author described that when antigen and antibody were placed in wells cut in a gel and allowed to diffuse, visible precipitin lines were formed at the zone of equivalence. By a system of serial dilution of test samples, an approximate concentration or titre could also be derived, as reported by the authors

Grabar and Williams (1953) described the technique of immunoelectrophoresis. Immunoelectrophoresis combined the advantage of zonal electrophoretic separation of proteins and the immunological discrimination of double diffusion.

Mancini et al (1965) described Single Radial Immunodiffusion, as a technique for quantitative estimation of proteins (antigens). Authors showed that the antigen diffused radially from the point of application into an antibody containing gel and a circular precipitate (ring) was formed at the zone of equivalence. Keeping antibody concentration and gel thickness constant; the area covered by precipitin ring was proportional to the concentration of antigen. In the original method, authors allowed the antigens to diffuse at room temperature until the precipitin rings stopped growing in size.

Fahey and McKelvey (1965) modified Mancini's technique of Single Radial Immunodiffusion. They reported that the readings could be taken after a fixed time viz. 18-20 hours; giving rise to only minute differences in the results.

Laurell (1966) described the technique Rocket Immunoelectrophoresis, a simple, quick and reproducible method for determination of a single protein in a protein mixture using number of samples simultaneously. Author applied diluted samples in wells side by side in a layer of agarose gel containing a monospecific antiserum. The identification of the protein was given by the rocket-shaped precipitate formed and quantitation was done by measuring the height of the precipitate rocket or the area under it.

XII- Demonstration of Complement Activation Products:

During last few years, direct methods for the demonstration of complement activation or breakdown products have become popular.

Lachmann and Coombs (1968) found that the titre of immunoconglutinin in serum, an antibody to reacted C3 and C4, was a measure of the extent of, in vivo, complement activation.

Thompson (1977) described a simple and reliable technique, known as 'Two Dimensional or Crossed Immunoelectrophoresis'. Author based this

test on the principle that both C3 and its activated fragment shared the same antigenic determinant but had different electrophoretic mobilities.

IV- Miscellaneous Method :

Fust (1978/1979) observed that deposition of complement components in various parts of renal glomeruli was of special importance in the diagnostics of certain renal diseases. Author also noticed that these deposits were made visible by immunofluorescence.

SIMPLIFIED SCHEME FOR EVALUATION OF COMPLEMENT :

Spitzer (1977b) gave a simplified scheme for evaluation of complement associated disorders by three screening tests including CH_{50}, C3 and C4 assays (Fig.:3).

PCM AND COMPLEMENT :

Smythe et al (1971) were the first to call attention to altered total haemolytic complement (CH_{50}) activity in infants with PCM. Estimation of haemolytic complement by these authors showed that 61% of infants with PCM had values well below 1/64 and 39% had values within normal limits, whereas the controls were consistently within a range of 1/128 to 1/512. These differences were found to be statistically significant.

Chandra (1978) first estimated the serum levels of complement component C3 in malnourished children by Mancini's single radial immuno-diffusion

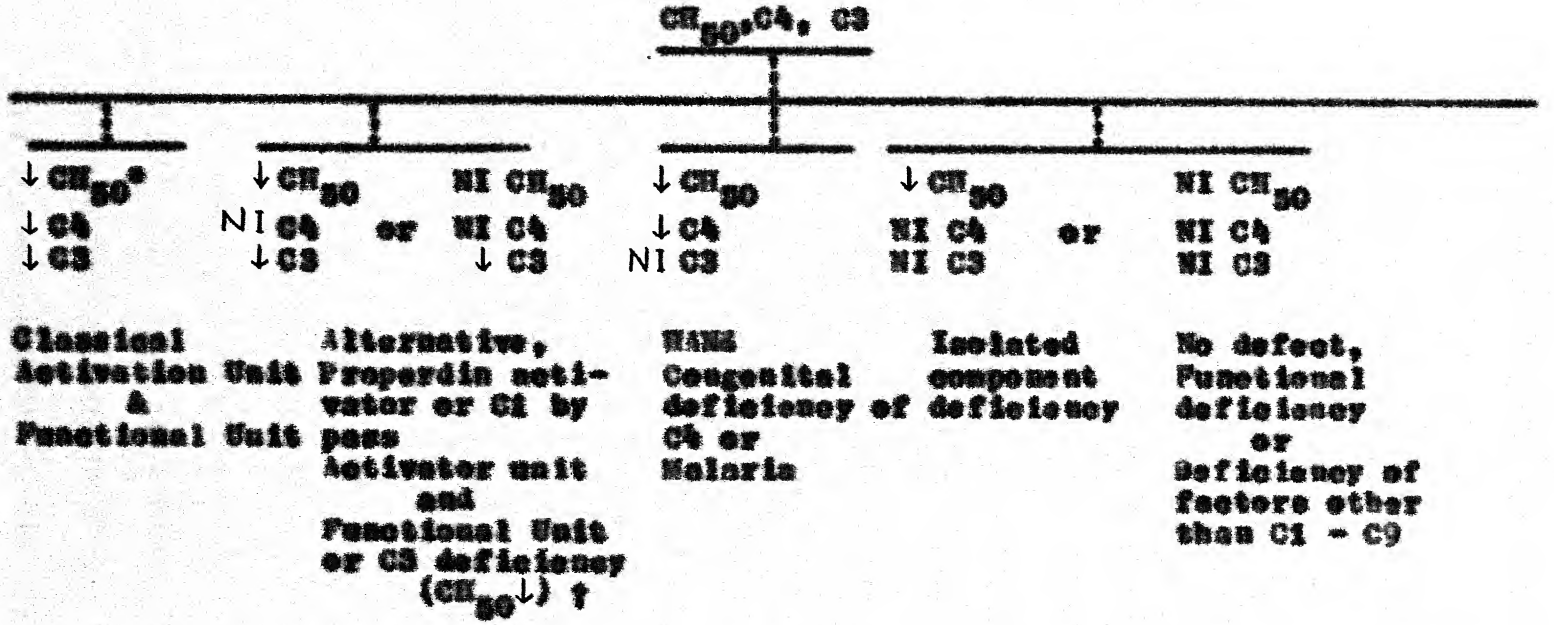

Fig. 3 : Simplified scheme for evaluation of complement-associated disorders; NI, Normal; *, Can be normal; †, differentiated by assays for C1, factor B, and properdin or properdin convertase.

technique. Serum levels of C3 were significantly lower in the malnourished children (98 mg% ± 32 S.D.) than in the control subjects (136 mg% ± 31 S.D.). He also suggested that this could well be the result of reduced synthesis by the liver cells.

Sirisinha et al (1973) studied the serum levels of complement proteins C1q, C1s, C3, C4, C5, C6, C8, C9 and C3 proactivator (C3 P.A.) in twenty children with PCM on admission and at intervals thereafter during different dietary treatments and compared the results with those in sixteen normal children of the same age in the same geographical area. A majority of patients were judged to be infected on admission and were placed on antibiotic therapy. Authors also found that the serum levels of all the complement proteins except C4 were markedly lower in malnourished children than in the normal children, and children with kwashiorkor had lower complement levels than those children suffering from marasmus. Admission levels of 8 of the 9 components (except C4) were slightly lower in the severely infected compared with the mildly infected patients as observed by the authors. Further the difference between the two groups was less pronounced on day 8, when infection seemed to be under control. However, the differences in the complement levels between severely infected and mildly infected patients were smaller than those between the malnourished and the normal children. These results

suggested that the poor diet associated with impaired synthesis of complement components and to a lesser extent, infections led to reduced serum complement levels in untreated PCM children. During follow up the quality of dietary protein and the calorie intake had a pronounced influence on the repair of the complement system, the best response was obtained by high calorie (175 C/Kg/day) and high protein diet (4 g/kg/day). The levels of most complement components during treatment rose to above normal values. Mechanism of this'overshoot' or 'rebound' should be due to accelerated synthesis after increased complement consumption in vivo as suggested by authors. They also suggested that, in addition to complement consumption, correction of impaired synthesis could result in complement 'rebound'.

In view of above observations, Chandra (1975) again subjected 25 children, aged 6 months to 4 years, having PCM to complement study and matched with 20 healthy controls. He observed that in 12 children there was clinical and microbiological or radiological evidence of systemic infection. These were treated with appropriate antibiotics. Three to eight weeks later a second sample of blood was drawn from 10 children, available for re-examination and no longer under-nourished. He noticed that total hemolytic complement and C3 concentration were significantly decreased in

malnourished children than that of healthy controls. There was also a significant positive correlation between C3 concentration and CH_{50} activity (r = 0.7131). Author observed a greater reduction in complement levels in malnourished children with infection compared with noninfected ones. However, in nutritionally normal subjects, infection was associated with high C3 levels. Author expressed the opinion that reversible but profound disturbance of complement seen in infected undernourished patients could be the result of at least two factors. One, antibody synthesis and cell division might get priority over complement synthesis in the face of limited nutrient resources of the host. Secondly, infection might be associated with complement consumption. Presence of second phenomenon, operating in these patients was supported by the fact that electrophoretically altered forms of C3 in 14 cases and raised levels of immunoconglutinin were detected in most of the cases. Finally the author suggested that reduced complement function in malnutrition was the combined result of impaired synthesis, complement activation in vivo, change in plasma volume, protein losing gastroenteropathy, and that it might contribute to an increased susceptibility to infection in undernourished individuals.

Neuman et al (1978) studied 76 malnourished Ghanaia children, aged 6 months to 6 years, and 41

age matched controls. Cases were divided into three groups :- Group I - Severely malnourished (34) children whose weights were 51-60% of 50th percentile of Harvard standard and /or serum albumin levels below 2.5 gm%. These children formed two subgroups - Kwashiorkor (23) and marasmus (11). Group II - Moderately malnourished, included 43 children whose weights were 61-80% of standard and serum albumin levels greater than 2.5 gm% and had minor skin and hair abnormalities. Lastly Group III - Control group consisted of children whose weights were 78% of standard, had normal serum albumin levels and were free of chemical signs of malnutrition or obvious infection except for pyoderma in a few cases. However, intestinal parasites were found in most of these cases. Authors noticed that levels of complement C3 were significantly reduced in the severely malnourished group as compared to the other two groups. Mean C3 levels in group II and III were slightly reduced but in a low normal range when compared to normal American children. In group I, children with kwashiorkor had lower C3 levels (mean 86 $\pm^{5}$ mg/100 ml) as compared to children suffering from marasmus (mean 71 $\pm$ 7 mg/100 ml). After 2 weeks of nutritional therapy mean C3 level in group I children with kwashiorkor increased to 78 $\pm$ 3 mg/100 ml. C4 levels were found to be normal in all 3 groups. Author explained that decreased C3 levels could be due to diminished protein synthesis by the liver as suggested

by a good correlation between the degree of C3 depletion and severity of depletion of other proteins. They also did not rule out the possibility of accelerated consumption as a result of infection occuring in these cases. Normal C4 level suggested that the alternative pathway of complement was activated in malnutrition probably by bacteria and their endotoxins which led to breakdown of C3 and later components without affecting C1, C2 and C4.

Using the haemolytic complement (CH_{50}) assay, Suskind et al (1976) evaluated the complement system of 38 children with severe PCM during their hospital admission and recovery. Children were classified clinically as having marasmus (M), marasmus-kwashiorkor (MK), and kwashiorkor (K) based on the scoring system of McLaren et al (1967). The mean CH_{50} activity in children with kwashiorkor was significantly less on hospital days 1 and 4 than in 17 healthy control subjects. On day 8 it rose to normal, and by day 30, it was significantly higher than controls. The mean CH_{50} titre of 16 well nourished febrile children was, in contrast to that of untreated PCM, significantly greater than in the healthy controls. Therefore it was unlikely that fever present in many PCM children, lowered their CH_{50} activity. Among children with PCM, 11 (40%) had detectable serum anticomplementary (AC) activity in their serum on either day 1 or 4. Significantly, the CH_{50} titre in a PCM serum correlated inversely with the amount of AC activity in the serum.

These results indicated that, in children with PCM, complement system was compromised functionally, and that its repair coincided with the intake of adequate diet. Further, presence of AC activity provided a possible explanation for depressed complement activity in some untreated PCM children.

Complement components C1-C9 were also estimated in children with protein-calorie malnutrition by Olusi et al (1976). Concentrations of C1q, C1s, C3, C6 and C9 were significantly lower in children with PCM, than in age and sex matched control children as observed by the authors. Children with marasmus tended to have higher values of these complement components than children with kwashiorkor. Complement C3 and C9 were the most severely affected by malnutrition and it would appear from the study, that more severe the degree of malnutrition, as judged by clinical examination and serum transferrin concentration, greater was the reduction in the serum concentration of C3 and C9. It was observed that the serum concentrations of C3 and C9 were lower in kwashiorkor and marasmic children with infections than in children without infections. There was no correlation between C3 and IgG concentrations as reported by the authors. It was suggested that probable responsible factors for reduced complement activity in malnutrition were reduced protein synthesis and increased utilization due to concomitant infections.

It was significant to observe in this series that there was no change in C4 concentration in children with malnutrition. It would appear that C4 was synthesized by the same cells responsible for the production of IgG and hence that there was a preferential synthesis of C4 and IgG in children with PCM. C5 was the only complement component which was significantly higher in malnourished children than in normal children, thus suggesting that this complement component was an acute phase protein. During refeeding, C3 was the first complement component to show a significant rise in concentration; this was followed by C9 and then C6. There was no change in C4 concentration while the levels of C5 fell. A conclusion drawn from these observations was that, of all of the complement components, C3 was the most sensitive index of nutritional status.

Kielmann et al (1976) carried out first ever study in nonhospitalized pre-school children in nine villages of the former Narangwal Rural Health Research Centre in Ludhiana District, Punjab. In these villages, all children upto 5 years of age routinely received curative and preventive medical facilities besides food supplement. Authors divided the children into 3 groups based on weight for age. These groups corresponded approximately to 80% or higher, 65% to 79% and less than 65% of the Harvard median, respectively. The children had significantly lower complement levels

(for all the three groups) as compared to those of reference population of identical age distribution. Children in the lowest weight for age group had less than 50% and those in the two higher nutritional groups had between 60% and 70% of the complement levels as compared to the reference population. Complement C3 levels were also positively correlated with several other anthropometric indices (weight-chest circumference and arm circumference for age) as observed by the authors.

Spitzer (1977a) mentioned that in patients with malnutrition, a consideration of failure of synthesis of C3 might be entertained. Decreased C3 levels could be used for diagnosis and also for monitoring during follow up.

Haller et al (1978) measured the plasma levels of complement haemolytic activity (CH_{50}), of some complement components and of C3d, a C3 break-down product, in 89 African children with various types of PCM including kwashiorkor, before and during recovery and compared them with two control groups, each consisting of ten age matched children and having a weight-age ratio of 790% of the Harvard standard. One of the control group was suffering from infection at the time of admission and the other had none. A significant decrease of CH_{50}, C3, C9 and factor B was observed in PEM. The decrease of CH_{50}, C3 and C9 appeared to be correlated with the severity of PEM, which was not the case for factor B.

On the other hand, levels of C4, C5 and C1 - Inactivator fell within normal range. Increased plasma levels of C3d with higher C3d/C3 ratio were also found in PEM patients as compared to non-malnourished infected patients and to normal non-infected children. Serial measurements done during the recovery of PEM indicated a progressive normalisation of all complement values, as well as a decrease of C3d/C3 ratio. Presenting their conclusions authors thought that two mechanisms could possibly be involved in the impairment of complement system in PEM : (1) a decreased synthesis of at least C3 and C9, as suggested by a significant correlation of C3 and C9 levels with those of serum albumin and cholinesterase; (2) an increased catabolism of C3, possibly due to an activation of the alternative complement pathway, as suggested by the increased levels of C3d and decreased level of factor B both of which were significantly correlated with C3 levels but not with albumin levels. Again C4 levels were normal as observed by authors.

Kelmann and Gurcio (1979) observed C3 complement levels in 83 rural pre-school children of North India. They related C3 complement levels to various parameters of nutritional status and past episodes of infections. All children were normally active and free from intercurrent infections. Mean complement levels were 28% lower than those found in an age-matched European

reference population. Low complement (C3) levels were associated mainly with children who were both stunted and wasted, as well as with those who had experienced frequent purulent skin infections in the past.

According to Johnston (1979) patients with malnutrition could have significant depletion of complement components and functional activity of complement. Although synthesis of components was depressed in malnutrition, serum from some patients also appeared to contain immune complexes which could accelerate depletion.

In a recent study Jagadeesan and Reddy (1979) reported that total haemolytic complement (CH_{50}) as well as C3 levels were significantly decreased in children with kwashiorkor (some of them had associated infections) and returned to normal after 3-4 weeks of treatment with protein and calories. In marasmic children, though the total complement activity was not significantly altered, C3 levels were reduced. However, neither CH_{50} nor C3 levels were found to be altered in mild to moderate protein-energy malnutrition (weight between 60-80% of standard). Reduction in serum complement activity could be one of the factors responsible for the frequent occurrence of infections in children with severe PEM as suggested by authors. Their study also indicated that immune status was not affected by milder degrees of PEM.

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MATERIAL & METHODS

The present study was carried out in the Department of Paediatrics, M.L.B. Medical College, Jhansi, in collaboration with the Department of Pathology, M.L.B. Medical College, Jhansi, over a period of 11 months from May 1981 to March 1982. Pre-school children (1-5 years age), attending the Well Baby Clinic and those admitted in the Paediatric ward, were selected for this study. Cases were grouped as :

A Healthy normal controls.

B Children suffering from protein-calorie malnutrition (PCM).

SELECTION OF CONTROLS :

Twelve normal healthy pre-school children were taken as control. Criteria for selection of control cases were :

1- Weight more than 80% of the 50th percentile of Harvard standard for age.

2- Exclusion of all possible factors known to affect the complement status viz. infections, liver and renal disorders, immunodeficiency diseases and corticosteroids.

SELECTION OF CHILDREN SUFFERING FROM PCM :

Thirty two children having PCM were taken for the present study. Criteria for selection of PCM cases were :

1. Weight less than 80% of the 50th percentile of Harvard standard for age.

2. Children having primary liver disorders, renal disorders and immunodeficiency diseases were excluded from the study.

3. No case was receiving corticosteroids.

Children suffering from PCM were treated with broad spectrum antibiotics, intravenous fluids, supplemental vitamins and minerals as per the requirements. All patients were put on nutritional rehabilitation schedule to raise the daily intake of food to more than 100 calories/kg along with 3-4 gm proteins/kg of the expected body weight.

An attempt was made to follow the cases at 2 weeks, 4-7 weeks and 10-12 weeks interval.

Besides name, age, sex, address and socio-economic status following facts were recorded in each case :

DIETARY HISTORY :

Dietary history was recorded with special emphasis on the following points :

a) The age upto which breast milk was given.

b) Age at which artificial milk was started.

Type of artificial milk and the quantum of dilution were also recorded.

c) Age at which semisolids and solids were started, was also noted.

d) Present diet in terms of quantity and quality of food material used in feeding the child was recorded. Total caloric and protein intake per day were thus recorded in every case to ascertain the cause of PCM.

IMMUNISATION STATUS :

History of immunisation was taken from the parents or family members. For small pox and BCG vaccination confirmation was made by careful inspection of scar marks. For polio and DPT vaccination, however, verbal statements were relied upon, confirmation was done by records, if available.

ANTENATAL, NATAL AND POSTNATAL HISTORY :

To rule out any secondary factor which could give rise to malnutrition, relevent antenatal, natal and post natal history was recorded. Special emphasis was also given to birth weight and gestational age of the child.

MILE STONES :

Mile stones were recorded under 4 headings : motor, manipulative, social and speech. The age, at which the child attained them was ascertained, by objective and subjective assessment.

PRESENT, PAST AND FAMILY ILLNESSES :

Present ailment relating to various systems were recorded.

Efforts were made to find out the occurrence of any acute or chronic illness in the past, that might have affected the nutritional status of the child. Past illness was mainly recorded in two categories : Category i - History of acute illness viz., fever, vomiting, diarrhoea and convulsion, lasting more than 4 days during the previous two weeks; category ii - History of cough, fever, vomiting, convulsions and diarrhoea, lasting more than 2 weeks any time during the previous 6 months. Besides these, definite history of primary complex, pertussis, measles or worm infestation was also recorded.

An enquiry was made about the history of any familial illness such as tuberculosis and diabetes.

PHYSICAL EXAMINATION :

A thorough clinical examination was made including those related to psychomotor changes, pallor, oedema, skin changes, hair changes, amount of subcutaneous tissue and muscle mass. Eyes were examined for the presence of xerosis and Bitot's spots. Skin was examined for any evidence of xerosis, hypopigmentation, hyperkeratosis and any dermatosis. Lips, gums and tongue were examined for the presence of angular lesions, cheilosis, gum swelling and glossitis.

Skeletal system was examined for the presence of any deformity and signs of vitamin D deficiency such

as craniotabes, cranial bossing, persistent open anterior fontanelle, costo-chondral beading and epiphyseal widening. Thyroid gland was examined to find out any abnormality.

Thorough systemic examination was made to detect any abnormality in cardiovascular, digestive, respiratory and central nervous systems.

ANTHROPOMETRIC MEASUREMENTS :

Weight

Weight was recorded nearest to 0.1 kg by using adult type weighing machine. For children who could not stand, Infant Weighing scale was used, capable of measuring weight to the nearest 0.05 kg. Same machines were used for subsequent follow up, to minimise the error.

Length/Height

Recumbent length was measured by an Infantometer and standing height was taken by a locally fabricated Stadiometer. These measurements were recorded nearest to 0.1 cm.

Mid-Arm Circumference

Circumference of left upper arm at the point midway between the tip of the acromion process of scapula and Olecranon process of ulna, was measured, while arm was hanging freely, to the nearest 0.1 cm. A flexible steel tape was used to record this.

Laboratory investigations viz. haemoglobin, leucocyte count (total and differential), total serum

proteins, serum albumin, urine and stool examinations were carried out routinely in each and every case. Radiological and other relevant investigations were performed if necessary.

Blood was collected by venepuncture. Samples of sera were harvested and stored frozen at -20° until ready for assay, but never for more than 4 months.

I - DETERMINATION OF TOTAL HAEMOLYTIC COMPLEMENT (CH_{50})

LEVEL :

Total haemolytic complement was determined by the technique of Mayer (1961).

Principle :

Measurement of total serum haemolytic complement is a useful screening test for the integrity of complement system. The test is based on the ability of sheep red cells, properly sensitised by rabbit antibody to sheep erythrocytes, to lyse in the presence of all 9 classical pathway components. Haemoglobin released by such lysis can be measured spectrophotometrically with great precision and related to the percentage of cells lysed.

Amount of complement required to lyse 50% SRBC constitutes one unit of CH_{50}. Complement titre is defined as the number of CH_{50} units contained in 1 ml of serum.

Material :

1. Alsever's Solution

Glucose 24.6 gm, trisodium citrate (dihydrate) 9.6 gm and sodium chloride 5.04 gm were dissolved in 1200 ml distilled water. The pH of solution was adjusted to 6.1 with 10% citric acid. It was then sterilized by low pressure autoclaving and stored in refrigerator. This solution was prepared fresh every 4 weeks. One volume or more was used for each volume of whole blood.

2. Stock Veronal Buffered Saline (Stock VBS)

A concentrated (5 times) solution was prepared by dissolving sodium chloride 85.0 gm, sodium 5,5 diethyl barbituate 10.19 gm in 1.5 litres of distilled water. The pH of solution was adjusted to 7.35 ± 0.05 with 1 N HCl and volume was made upto 2.0 litres. This solution was stored for 1 month at 4°C.

3. Isotonic Gelatin Veronal Buffered Saline (GVBS)

One part of stock VBS was mixed with 4 parts of distilled water. Sufficient dry gelatin was added to give final gelatin concentration of 0.1%. Gelatin was dissolved by gently heating and mixing the solution.

One ml each of 0.5 M $CaCl_2$ and 2 M $MgCl_2$ were added to each 1 litre of GVBS. This solution was prepared fresh every week.

4. Antisheep Haemolysin (procured commercially).

5. Normal Human Serum (NHS).

This was used as a source of complement (procured from a healthy donor).

6. Sheep Red Blood Cells (SRBC).

These were procured from jugular vein of a healthy sheep with the help of a dry sterilised syringe.

7. Test Serum.

(This was collected from the patient under investigation.)

Procedure :

1. Preparation of Sheep Red Blood Cells (SRBC) Suspension.

SRBC were collected in Alsever's solution and used 3-5 days after collection and within 15 days of collection. SRBC were stored at 4°C.

On the day of the test sheep erythrocytes were washed thrice in GVBS. One volume of the packed cells was suspended in 19 volumes of buffer to give a slightly greater than 5% suspension. One ml of this suspension was lysed with exactly 14 ml of distilled water and the optical density (O.D.) was measured at 541 nm with distilled water as blank.

A lysate with O.D. of 0.7 was considered to contain 5% or $1x10^9$ Cells/ml. From the O.D. of the sample tested and volume of the suspension (V1), final volume (Vf) to which the suspension was

adjusted to make a standardized suspension, was calculated according to the relationship :

$$Vf = \frac{Vi \times O.D.}{0.7}$$

2. Titration of Haemolysin

This was first performed so that the complement titration was independent of the concentration of haemolysin. 5.0 ml volumes of 5% SRBC were treated with equal volumes of 1:50, 1:100, 1:200, 1:400 and 1:800 diluted haemolysin in GVBS for 15 minutes at 37°C. This suspension of sensitized SRBC was now called EA.

6.5 ml volumes of 1:50, 1:100, 1:200 and 1:400 diluted normal human serum (NHS) were also prepared and taken in tubes A to D in 5 sets.

In tubes E and F, 6.5 ml. each of GVBS and distilled water were taken, respectively. Then 1 ml. of E A 1:50 was poured in 1st set of tubes from A to F. The same procedure was repeated for EA 1:100, 1:200, 1:400 and 1:800 for rest of 4 sets of tubes.

	NHS (1:50) 6.5 ml A	NHS (1:100) 6.5 ml B	NHS (1:200) 6.5 ml C	NHS (1:400) 6.5 ml D	GVBS 6.5 ml E	Distilled water 6.5 ml F
EA (1:50) 1ml						
EA (1:100) 1ml						
EA (1:200) 1ml						
EA (1:400) 1ml						
EA (1:800) 1ml						

After mixing the contents of tubes in each set, these were incubated at 37°C for 60 minutes. The tubes were then centrifuged and optical density (O.D.) measured at 541 nm. Percentage of haemolysis was calculated by the formula :

$$\text{Haemolysis (\%)} = \frac{\text{O.D. Row A to 5} - \text{O.D. Row E}}{\text{O.D. Row F}} \times 100$$

Dilution factors regarding antiserum (used in EA suspension) and normal human serum were read and used for further titration. These dilutions were found to be 1:100 and 1:50 respectively in the present series.

3. Titration of Complement

5% suspension of SRBC was prepared. Equal volume of 1:100 diluted haemolysin was added to SRBC suspension. This mixture was then incubated at 37°C for 15 minutes and was stored in a refrigerator till use (1 to 2 hours usually).

Test serum was diluted to 1:50 in GVBS and titration was set up as follows :

TUBE	1	2	3	4	5	6
GVBS ml	4.0	3.0	1.5	0.5	6.5	-
Distilled water ml	-	-	-	-	-	6.5
EA ml	1.0	1.0	1.0	1.0	1.0	1.0
Test serum (1:50) ml	2.5	3.5	5.0	6.0	-	-

After mixing the contents of tubes in each column, the test material was incubated at 37°C for 60 minutes. Then tubes were centrifused and optical density of the supernatent in each tube was read at 541 nm with distilled water containing tube as a blank.

4. Calculations to Determine the Number of Units of Total Haemolytic Complement per ml of Serum (CH_{50})

Haemolysis (Y) was calculated for each tube as follows :

$$Y = \frac{\text{O.D. tube 1 to 5} - \text{O.D. tube 5}}{\text{O.D. tube 6}}$$

A graph was plotted. The log of the amount of test serum added (log X) was plotted on the abscissa; log $\frac{Y}{1-Y}$ was plotted on the ordinate. The antilog of X where straight line crossed 0 (zero) on the ordinate gave the volume of test serum needed for 50% lysis.

CH_{50}U/ml of undiluted serum was calculated as follows:

$$CH_{50}U/ml = \frac{\text{dilution of serum}}{\text{volume required for 50\% lysis}}$$

II- DETERMINATION OF ALTERNATIVE PATHWAY ACTIVITY(AP_{50}):

Alternative pathway activity was assessed by the technique described by Platts-Mills and Ishizaka (1974).

Principle :

Unsensitized rabbit erythrocytes (RBC) activate the alternative pathway of complement in

normal human serum. Hence the lysis of RBC is used to assess the functional activity of the alternative pathway components.

Material :

1. Alsever's Solution.
2. Stock Veronal Buffered Saline (Stock VBS)
3. Isotonic Gelatin Veronal Buffered Saline (GVBS)

For alternative pathway activity, only 1 ml of 2 M $MgCl_2$ was added to each 1 litre GVBS. This was prepared fresh every week.

4. Stock EDTA

Disodium ethylene diamine tetra acetate 37.2 gm was dissolved in 800 ml distilled water. The pH was adjusted to 7.68 ± 0.05 with freshly prepared 2 M NaOH and volume was made to 1 litre. This was stored for 2 weeks at 4°C.

5. GVBS - EDTA

Nine parts of isotonic GVBS (without $CaCl_2$ and $MgCl_2$) was mixed with 1 part of stock EDTA. This was prepared fresh every week.

6. Rabbit Red Blood Cells. (These were procured by giving a cut on the ear margin of a healthy rabbit and blood collected under sterile conditions).
7. Test Serum (This was collected from the patient).

Procedure :

RBC, collected in Alsever's solution, were used from day 0 - 15 of collection. These were kept in the refrigerator after collection.

On the day of the test a 2.5% suspension of RBBC in buffer was prepared in the same way as SRBC in the determination of CH_{50}.

Test serum was diluted to 1:45 in GVBS and titration was set up as follows :

TUBE		1	2	3	4	5	6
GVBS	ml	0.40	0.30	0.15	0.05	0.65	-
Distilled water	ml	-	-	-	-	-	0.65
RBBC	ml	0.10	0.10	0.10	0.10	0.10	0.10
Test serum (1:45)	ml	0.25	0.35	0.50	0.60	-	-

After mixing the tubes in each column, test material was incubated at 37°C for 30 minutes. Reaction was stopped by adding 3 ml of GVBS - EDTA. Tubes were then centrifuged and optical density (O.D.) of the supernatant was measured at 412 nm.

Calculation was done exactly in the same way as for CH_{50} and expressed as AP_{50} U/ml.

III- <u>DETERMINATION OF C3 CONCENTRATION</u> :

Serum C3 levels were estimated by single radial immunodiffusion technique as described by Mancini et al (1965), with suitable modifications according to Pahey and McKelvey (1965).

Principle :

Antigen diffuses radially from the point of application into an antibody containing gel and a circular precipitate (ring) is formed at the zone of equivalence. Keeping antibody concentration and gel thickness constant, the area covered by precipitation ring is proportional to the concentration of antigen.

Material :

1. Stock Barbitone Buffer (0.12 M, pH 8.6)

Sodium barbitone 20.6 gm and barbitone 4.0 gm were dissolved in distilled water to a final volume of 1 litre with pH 8.6. One gm sodium azide was added per litre as a preservative.

2. Working Barbitone Buffer (0.05 M)

Stock was diluted to 1 : 2.

3. Monospecific Antisera Against C3 (anti-C3)

(This was procured commercially).

4. Standard Normal Human Serum with Known Amount of C3

(This was procured commercially).

5. Unknown Test Serum

(This was obtained from the patient).

6. Glass slides of size 7.5 x 5.0 cm, gel punch, moist chamber, Immunomeasure.

Procedure :

Two percent Agar gel was prepared in working barbitone buffer. 2.5 ml volume of melted agar in a test tube was kept in a water bath maintained at 45°C.

2.5 ml of anti-C3 diluted 1:8 in buffer was added to the tube containing 2.5 ml agar and mixed thoroughly and poured over a clean glass slide kept on a horizontal table. Air bubbles, whenever present, were removed with a hot wire loop. Gel was allowed to set for 10-15 minutes.

Fifteen evenly spaced wells, of 3 mm size each, were cut, with the help of a punch using a predesigned template.

Measured volume (5 ul each) of various dilutions of standard serum and appropriately diluted test sera, were run into the wells. The plate was then incubated in a moist chamber at room temperature and diffusion was allowed for 20 hours. Finally the diameter (d) of precipitin rings was measured by immunomeasure and d^2 (diameter square) was obtained in mm (Fig.: 4).

A standard curve was drawn by plotting d^2 vs known concentrations of standard serum. Concentrations of unknowns (with known diameter) were obtained from the curve. Final results were obtained after multiplying the readings with the dilution factor and expressed in mg/dl.

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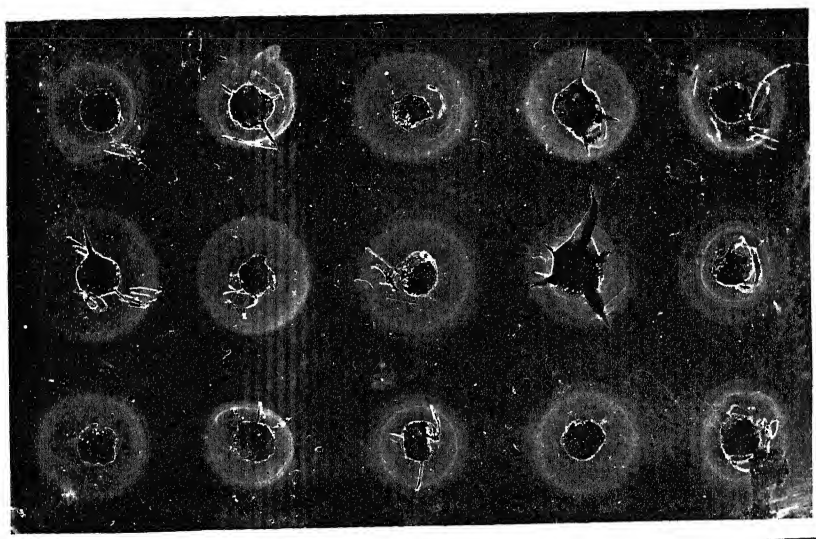

Fig.4 : Showing Single Radial Immunodiffusion.

OBSERVATIONS

A study to assess the functional activity of complement system in protein-calorie malnutrition(PCM) was carried out in 32 pre-school children(1-5 years age) at M.L.B. Medical College, Jhansi, between May 1981 and March 1982. Besides complement activity, various anthropometric measurements, serum albumin and blood haemoglobin values were noted in each case. A control group, comprising 12 age matched well nourished children, was also studied for comparative evaluation of complement activity, anthropometric measurements, serum albumin and haemoglobin values.

Age distribution of control and PCM cases is shown in Table I.

TABLE I

Age distribution of control and PCM cases.

Clinical group	Age distribution (months)				Total
	12-24	24-36	36-48	48-60	
Control	5	4	1	2	12
PCM	18	9	4	1	32
Total	23	13	5	3	44

Cases of PCM, 32 in number were further divided into 3 clinical groups according to Wellcome classification. Based on this classification, 19 cases

had marasmus, 10 had marasmic-kwashiorkor and 3 were suffering from kwashiorkor (Table II).

Table II

Clinical groups of PCM according to Welaren classification.

Clinical group	Welaren score	No.of cases
Marasmus	0 - 3	19
Marasmic-kwashiorkor	4 - 8	10
Kwashiorkor	9 - 15	3
Total	15	32

All cases of PCM belonged to low socio-economic status and on admission most of them were suffering from various infections as demonstrated by clinical features, pathological and radiological investigations. Fifteen of these PCM cases had gastrointestinal symptoms; 6 were suffering from respiratory tract infections, while 9 cases had mixed picture of gastrointestinal, respiratory, skin, eye and ear infections. One case each had isolated Bell's palsy and Infantile Tremor Syndrome. Out of 32 PCM cases, 2 had severe fulminating infections in the form of staphylococcal pneumonia, severe gastroenteritis or pyodermia.

An analysis of the history of past illnesses revealed that 15 cases had combined features of gastro-intestinal and respiratory system involvement, while 22

cases had a definite history of either pertusis, measles or worm infestation.

Family history of tuberculosis was elicited in 6 cases.

All the cases, at the time of admission, were receiving diet, grossly deficient in calories as well as proteins.

None of the 12 control cases had a history of infections in the immediate past and they were not suffering from any demonstrable illness at the time of inclusion in this study.

I - INITIAL CONTACT :

1- Anthropometric Values :

Anthropometric profile of control and PCM cases at the initial contact is depicted in Table XII.

As is evident from Table, mean anthropometric values of weight, length (height) and mid-arm circumference in children suffering from PCM were appreciably less than in controls. However different groups of PCM, as per McLaren classification, did not reveal clear differences in the anthropometric measurements.

2- Serum Albumin, Haemoglobin Values and Complement Activity in Two Study Groups :

Mean serum albumin deemed to be significantly depressed in PCM (2.12 ± 0.09 gm/dl) as compared to controls ($P < 0.001$). Similarly mean haemoglobin value

Table III

Anthropometric profile in control and PCM cases

Clinical group	No.of cases	Age(months) Mean ± S D	Weight(kg) Mean ± S D	Length or height(cm) Mean ± S D	Mid-arm circumference (cm) Mean ± SD	Weight expressed as % of 50th percentile of Harvard Standard Mean ± S D	Length(height) expressed as % of 50th percentile of Harvard Standard Mean ± S D	Mid-arm circumference expressed as % of Tolunski Standard Mean ± S D
Control	12	32.42 ± 16.86	12.39 ± 3.88	87.71 ± 11.58	15.45 ± 0.78	89.63 ± 4.41	95.17 ± 2.82	94.61 ± 4.06
PCM	52	27.13 ± 12.26	6.72 ± 1.31	78.82 ± 6.97	9.93 ± 1.82	52.91 ± 8.88	84.18 ± 5.27	64.41 ± 9.34
Marasmus	19	28.84 ± 12.80	6.67 ± 1.34	74.79 ± 6.58	10.08 ± 1.47	51.88 ± 8.46	84.29 ± 5.66	62.14 ± 9.07
Marasmic-kwashiorkor	10	33.00 ± 6.88	6.59 ± 1.36	78.67 ± 7.87	9.74 ± 1.87	54.17 ± 8.86	84.95 ± 4.38	60.48 ± 9.90
Kwashiorkor	3	30.00 ± 16.70	6.97 ± 1.70	71.50 ± 7.86	9.93 ± 2.28	52.82 ± 3.41	80.33 ± 5.83	59.89 ± 12.60

(7.82 ± 1.62 gm/dl) was significantly lower in PCM as compared to controls ($P < 0.001$), as shown in Table IV.

Complement activity was evaluated by three important parameters viz. total haemolytic complement (CH_{50}U/ml), alternative pathway activity (AP_{50}U/ml) and C3 concentration (mg/dl).

Table IV shows that at the time of initial contact mean CH_{50} value in PCM group was 5.89 ± 3.45 U/ml and mean AP_{50} value was 64.66 ± 23.07 U/ml. A comparison of mean CH_{50} and AP_{50} values between control and PCM group revealed that the differences were not statistically significant ($P > 0.05$). However, mean serum C3 concentration (60.89 ± 23.26 mg/dl) in the PCM group was found to be significantly lower than in the control group ($P < 0.001$).

3- <u>Serum Albumin, Haemoglobin, CH_{50}, AP_{50} and C3 Values in Various Groups of PCM</u> :

Mean serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 values in various groups of PCM as per Wellcome classification viz. marasmus, marasmic-kwashiorkor and kwashiorkor are depicted in Table V.

Table IV

Serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 values in two study groups.

Clinical group	No.of cases	Serum albumin (gm/dl) Mean ± S D	Haemoglobin (gm/dl) Mean ± S D	CH_{50} (U/ml) Mean ± S D	AP_{50} (U/ml) Mean ± S D	C3 (mg/dl) Mean ± S D
Control	18	4.06 ± 0.27	12.57 ± 1.04	7.16 ± 1.92	64.70 ± 10.11	125.88 ± 23.98
PCM	32	3.12 ± 0.80	7.82 ± 1.62	5.39 ± 3.45	61.86 ± 23.07	60.59 ± 23.25
P value		$<$ 0.001	$<$ 0.001	$>$ 0.05	$>$ 0.05	$<$ 0.001

Table V

Serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 values in controls and various groups of PCM

	Control	Haramus		Haramic-kwashiorkor		Kwashiorkor	
	Mean ± S D	Mean ± SD	Signi-ficance vs control	Mean ± S D	Signi-ficance vs control	Mean ± S D	Signi-ficance vs control
Serum albumin (gm/dl)	4.06 ± 0.27	3.49 ± 0.85	< 0.01	2.72 ± 0.41	< 0.001	2.08 ± 1.62	< 0.001
Haemoglobin (gm/dl)	12.87 ± 1.04	8.62 ± 1.14	< 0.001	6.96 ± 1.60	< 0.001	5.60 ± 0.83	< 0.001
CH_{50} (U/ml)	7.16 ± 1.92	5.81 ± 3.88	> 0.05	5.77 ± 2.70	> 0.05	5.48 ± 2.96	> 0.05
AP_{50} (U/ml)	64.70 ± 10.11	63.82 ± 24.91	> 0.05	62.32 ± 22.83	> 0.05	51.40 ± 3.90	< 0.05
C3 (mg/dl)	125.00 ± 23.98	63.16 ± 23.98	< 0.001	62.30 ± 23.00	< 0.001	38.67 ± 2.31	< 0.001

Serum Albumin :

Mean serum albumin values (gm/dl) in marasmus, marasmic-kwashiorkor and kwashiorkor groups were 3.49 ± 0.83, 3.72 ± 0.41 and 2.03 ± 1.62 at the initial contact (Fig.5). These values were significantly lower as compared to controls (P < 0.01 in marasmus and < 0.001 in rest two groups). Mean serum albumin value was lowest in cases suffering from kwashiorkor, when first seen.

Haemoglobin :

Mean haemoglobin values (gm/dl) were found to be 8.62 ± 1.14, 6.96 ± 1.60 and 5.60 ± 0.82 in marasmus, marasmic-kwashiorkor and kwashiorkor respectively. These values were found to be significantly lower as compared to controls (P < 0.001). Mean haemoglobin percentage was the lowest in kwashiorkor cases as compared to other two groups.

CH_{50} :

Mean CH_{50} values (U/ml) in marasmus, marasmic-kwashiorkor and kwashiorkor groups at the time of first contact were 5.81 ± 3.08, 5.77 ± 2.70 and 5.48 ± 3.96 respectively (Fig.6). A comparison of these mean values with those in control subjects revealed that all 3 groups each had lower but not statistically significant mean values, as compared to control group (P > 0.05). Also the group differences of mean CH_{50} values were not appreciably different.

SERUM ALBUMIN CONCENTRATION IN CONTROL AND PCM

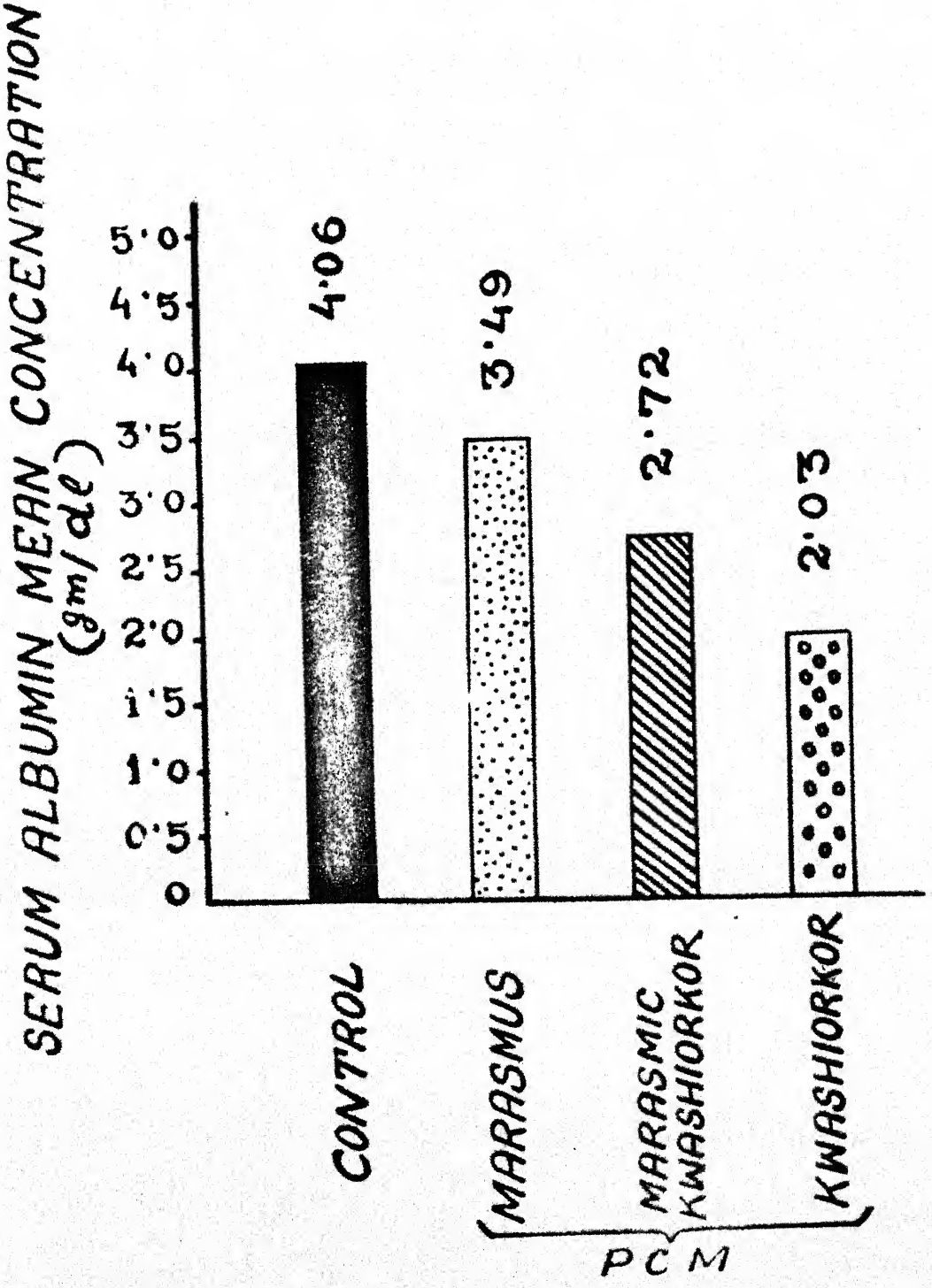

FIG. NO. 5.

TOTAL HAEMOLYTIC COMPLEMENT (CH_{50}) ACTIVITY IN CONTROL AND PCM.

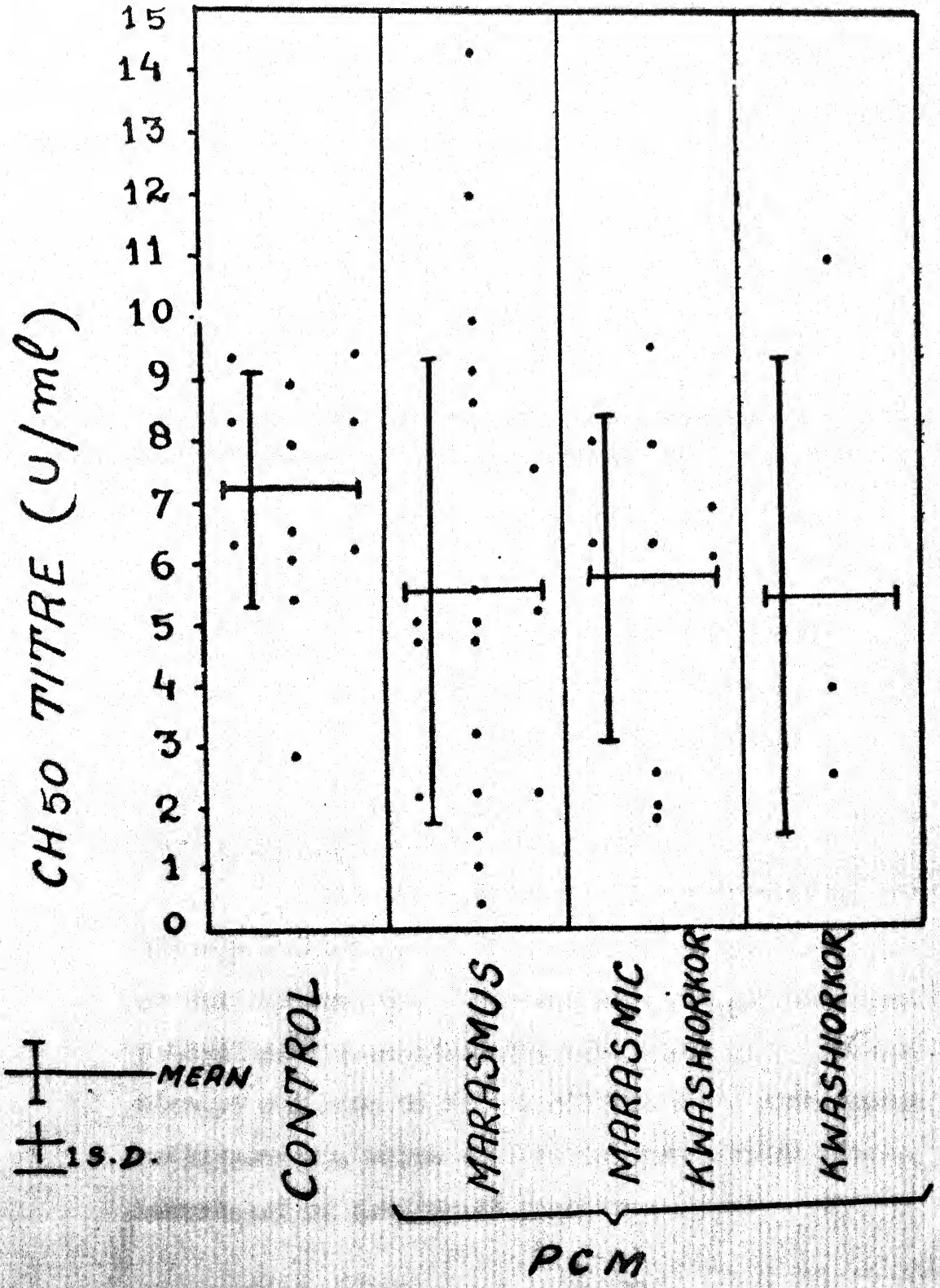

FIG. No. 6.

AP_{50}:

Mean AP_{50} values (U/ml) in marasmus, marasmic-kwashiorkor and kwashiorkor, on initial contact, were 63.82 ± 24.91, 62.22 ± 22.83 and 51.40 ± 8.90 respectively (Fig.:7). It is evident that mean values in three groups of PCM were lower as compared to controls. However, mean AP_{50} value in kwashiorkor group was maximally depressed and statistically significant ($P < 0.05$).

Complement C3 :

Mean C3 values (mg/dl) in marasmus, marasmic-kwashiorkor and kwashiorkor groups were found to be 63.16 ± 23.98, 62.30 ± 23.86 and 38.67 ± 2.31 respectively (Fig.: 8). These values were significantly lower as compared to control cases ($P < 0.001$). However, the mean values in 3 groups were not appreciably different from each other.

II- <u>FOLLOW UP</u> :

After the assessment of anthropometry, serum albumin and haemoglobin values, and complement activity on initial contact, PCM cases were put on nutritional rehabilitation schedule and infections were treated. An attempt was made to follow all PCM cases with repeat anthropometry, serum albumin and haemoglobin levels, and assessment of complement activity.

ALTERNATIVE PATHWAY ACTIVITY (AP_{50}) IN CONTROL AND PCM

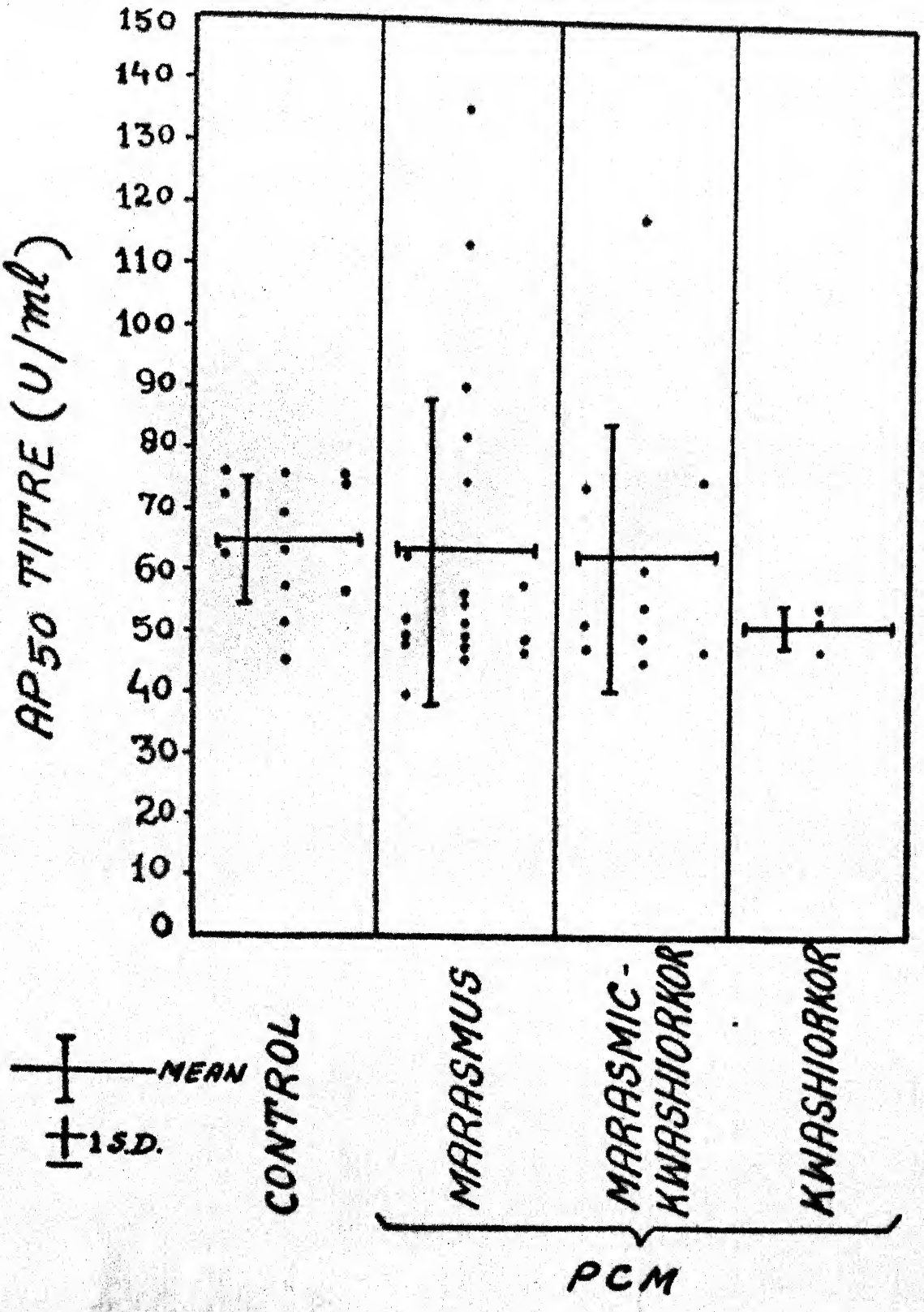

FIG. No. 7.

COMPLEMENT C3 CONCENTRATION IN CONTROL AND PCM

FIG. No. 8.

In first follow up after an interval of 2 weeks 17 cases could be taken; in 2nd follow up, after 4-7 weeks, 7 cases turned up and 3rd follow up could be done after 10-12 weeks in only 2 cases.

1. Anthropometric Profile :

Marasmus Group :

Anthropometric profile in the follow up groups of marasmus is shown in Table VI.

Table VI

Anthropometric profile in follow up groups of marasmus.

Clinical group	No.of cases	Weight(kg) Mean ± S D	Length(height) (cm) Mean ± S D	Mid-arm circumference (cm) Mean ± S D
Initial sample	19	6.67 ± 1.24	74.79 ± 6.58	10.03 ± 1.47
1st follow up	9	7.91 ± 1.05	75.39 ± 5.64	11.16 ± 1.31
2nd follow up	4	8.05 ± 1.66	74.18 ± 6.68	12.50 ± 1.31
3rd follow up	1	11.50	83.50	14.40

Table VI shows that improvement in weight and mid-arm circumference were sustained during follow up. Mean weight increased by 1.24 kg and 1.37 kg in the first and second follow up, respectively. Arm circumference showed an increase of 1.13 cm and 2.47 cm in mean values, after 1st and 2nd follow up respectively. A single case, who turned up on three occasions showed a consistent rise in weight from 8.00 kg at initial contact to 9.00 kg, 9.70 kg and 11.50 kg on 2nd, 3rd and 4th contact respectively.

Similarly arm circumference values in the same case improved from 11.80 cm at the initial contact to 12.40 cm, 14.20 cm and 14.40 cm at subsequent dates during follow up. However, improvement in mean length (height) did not seem to be constant during follow up period.

Marasmic-kwashiorkor Group :

Anthropometric profile in the follow up groups of marasmic-kwashiorkor is depicted in Table VII.

Table VII

Anthropometric profile in follow up groups of marasmic-kwashiorkor.

Clinical group	No.of cases	Weight(kg) Mean ± S D	Length(height) (cm) Mean ± S D	Mid-arm circumference (cm) Mean ± S D
Initial sample	10	6.89 ± 1.36	73.67 ± 7.67	9.74 ± 1.57
1st follow up	7	7.21 ± 1.04	74.34 ± 7.68	9.99 ± 0.77
2nd follow up	3	6.90 ± 0.40	68.67 ± 6.70	10.97 ± 0.80
3rd follow up	1	11.00	78.00	12.00

In marasmic-kwashiorkor group, gain in mean weight was 0.62 kg and 0.31 kg during 1st and 2nd follow up dates. However, one individual, who turned up on all three occasions, showed a weight increase of 1.15 kg, 1.55 kg and 3.35 kg respectively. Again in arm circumference values, there was a sustained improvement during 2 follow ups viz. 0.25 cm and 1.23 cm and an individual case

who had three repeat measurements showed an increase of 0.80 cm, 1.80 cm and 2.30 cm during 1st, 2nd and 3rd follow up respectively. As with marasmus, no constant improvement was noticed in length (height) measurement during the follow up.

Kwashiorkor Group :

Anthropometric profile in the follow up group of kwashiorkor is shown in Table VIII.

Table VIII

Anthropometric profile in follow up group of kwashiorkor.

Clinical group	No.of cases	Weight(kg) Mean ± S D	Length(height) (cm) Mean ± S D	Mid-arm circumference (cm) Mean ± S D
Initial sample	2	6.97 ± 1.70	71.50 ± 7.86	9.98 ± 2.38
1st follow up	1	7.90	67.00	12.00

In this group, only one case turned up at the first follow up. After a period of 2 weeks treatment on prescribed diet, weight showed an increase from initial 6.90 kg to 7.90 kg, subsequently. Length increased from 66.00 cm initially, to 67.00 cm after 2 weeks. Arm circumference increased from 11.30 cm to 12.00 cm after 2 weeks of nutritional rehabilitation.

2. Serum Albumin, Haemoglobin, CH_{50}, AP_{50} and C3 Values :

Marasmus Group :

Mean values of serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 are shown in Table IX.

Table IX

Serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 values in follow up groups of neonates.

Clinical group	No.of cases	Serum albumin (gm/dl) Mean ± S D	Haemoglobin (gm/dl) Mean ± S D	CH_{50} (U/ml) Mean ± S D	AP_{50} (U/ml) Mean ± S D	C3 (mg/dl) Mean ± S D
Initial sample	19	3.49 ± 0.88	8.68 ± 1.14	8.81 ± 2.88	68.82 ± 24.91	68.16 ± 33.88
1st follow up	9	3.91 ± 0.88	9.76 ± 1.08	10.24 ± 2.82	61.45 ± 8.13	135.32 ± 42.82
2nd follow up	4	3.97 ± 0.48	10.70 ± 0.89	8.64 ± 2.98	68.16 ± 10.21	164.00 ± 24.99
3rd follow up	1	4.40	11.00	7.98	86.68	114.00

Serum Albumin :

There was a sustained increase in mean serum albumin levels during first and second follow up periods, being 0.42 gm/dl and 0.48 gm/dl respectively. In a single case who turned up for 3rd follow up, 0.50 gm/dl rise in mean serum albumin level was observed. Thus a sustained increase in albumin levels was observed throughout the follow up, in wasteness cases.

Haemoglobin :

Like serum albumin levels, mean haemoglobin values increased from 8.62 gm/dl initially to 9.76 gm/dl and 10.70 gm/dl in 1st and 2nd follow up, respectively. At the 3rd follow up, Hb level increased from initial 7.80 gm/dl to 11.00 gm/dl in a single case observed.

CH_{50} :

From Table IX, showing the effect of nutritional repair on CH_{50} levels, it is evident that CH_{50} attained maximum mean level (10.24 U/ml) after 1st follow up as compared to initial mean of 5.51 U/ml; a rise of 4.73 U/ml in mean value. Although, in 2nd follow up, the mean level decreased (9.61 U/ml) yet it remained higher than the initial with a difference of 3.10 U/ml. A case who turned up for 3rd follow up showed that CH_{50} value decreased from 11.99 U/ml at initial contact to 7.93 U/ml at the last follow up.

AF_{50} :

On evaluation of AF_{50} values in follow up groups, it is clear that there was no constant difference in repeat values as compared to initial one.

Complement C3 :

There was a rise in mean serum C3 concentration after the 1st and 2nd follow up being 72.17 mg/dl and 100.84 mg/dl from the initial. However, in a single case, followed 3rd time, a rise of only 26 mg/dl in C3 value was observed from initial contact to last follow up (Fig.19).

<u>Maxamia-Exanthiestar Group</u> :

Mean values of serum albumin, haemoglobin, CH_{50}, AF_{50} and C3 are depicted in Table X.

Serum Albumin :

Table X reveals that between the initial contact and 1st follow up there was rise of 1.15 gm/dl in mean serum albumin level as compared to the initial sample. However, there was no further rise in the mean albumin level at second follow up. Maximum increase of albumin value i.e. 2.10gm/dl was observed in a single case after 3rd follow up.

Haemoglobin :

Mean Hb values showed a sustained increase during the 1st and 2nd follow up assessment, being 1.93 gm/dl and 3.61 gm/dl respectively. However, after 3rd follow up in a single case, there was a rise of only 1.30 gm/dl in haemoglobin from the initial value.

COMPLEMENT C3 CONCENTRATION IN INITIAL AND FOLLOWUP GROUPS OF MARASMUS

FIG. No. 9.

Table X

Serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 values in follow up groups of Marasmic-kwashiorkor.

Clinical group	No. of cases	Serum albumin (gm/dl) Mean ± S D	Haemoglobin (gm/dl) Mean ± S D	CH_{50}(U/ml) Mean ± SD	AP_{50}(U/ml) Mean ± S D	C3 (mg/dl) Mean ± S D
Initial sample	10	2.72 ± 0.41	6.96 ± 1.60	5.77 ± 2.70	62.32 ± 22.53	82.30 ± 25.88
1st follow up	7	3.67 ± 0.35	8.89 ± 0.92	9.23 ± 3.04	59.63 ± 11.88	123.14 ± 44.16
2nd follow up	3	3.87 ± 0.15	10.87 ± 0.86	7.20 ± 4.08	62.05 ± 15.46	112.00 ± 45.83
3rd follow up	1	5.10	10.60	6.29	65.12	142.00

CH_{50} :

It was observed that mean CH_{50} level raised by 2.46 U/ml after 1st follow up. Although mean CH_{50} decreased in 2nd follow up as compared to the value in 1st follow up, yet it remained higher by 1.43 U/ml than what it was at the initial contact. Maximum rise of 3.78 U/ml, was observed in a single case, between the initial contact and 3rd follow up.

AP_{50} :

As with marasmus group, there was no consistent pattern of rise in AP_{50} values during the follow up.

Complement C3 :

On evaluation of mean C3 levels, a rise of 60.84 mg/dl was observed during 1st follow up. Although after 2nd follow up mean value decreased, it still persisted at higher level than it was at the time of initial contact, the difference being of 49.70 mg/dl. Maximum increase in C3 concentration viz. 111.00 mg/dl was noticed in a single case between the initial contact and 3rd follow up (Fig.:10).

<u>Kwashiorkor Group</u> :

Serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 values are shown in Table XI.

In this group only one case could be followed up, after 2 weeks from the initial contact.

Serum Albumin :

Serum albumin value increased from 1.30 gm/dl initially, to 2.90 gm/dl after 2 weeks of nutritional therapy.

COMPLEMENT C3 CONCENTRATION IN INITIAL AND FOLLOWUP GROUPS OF MARASMIC KWASHIORKOR

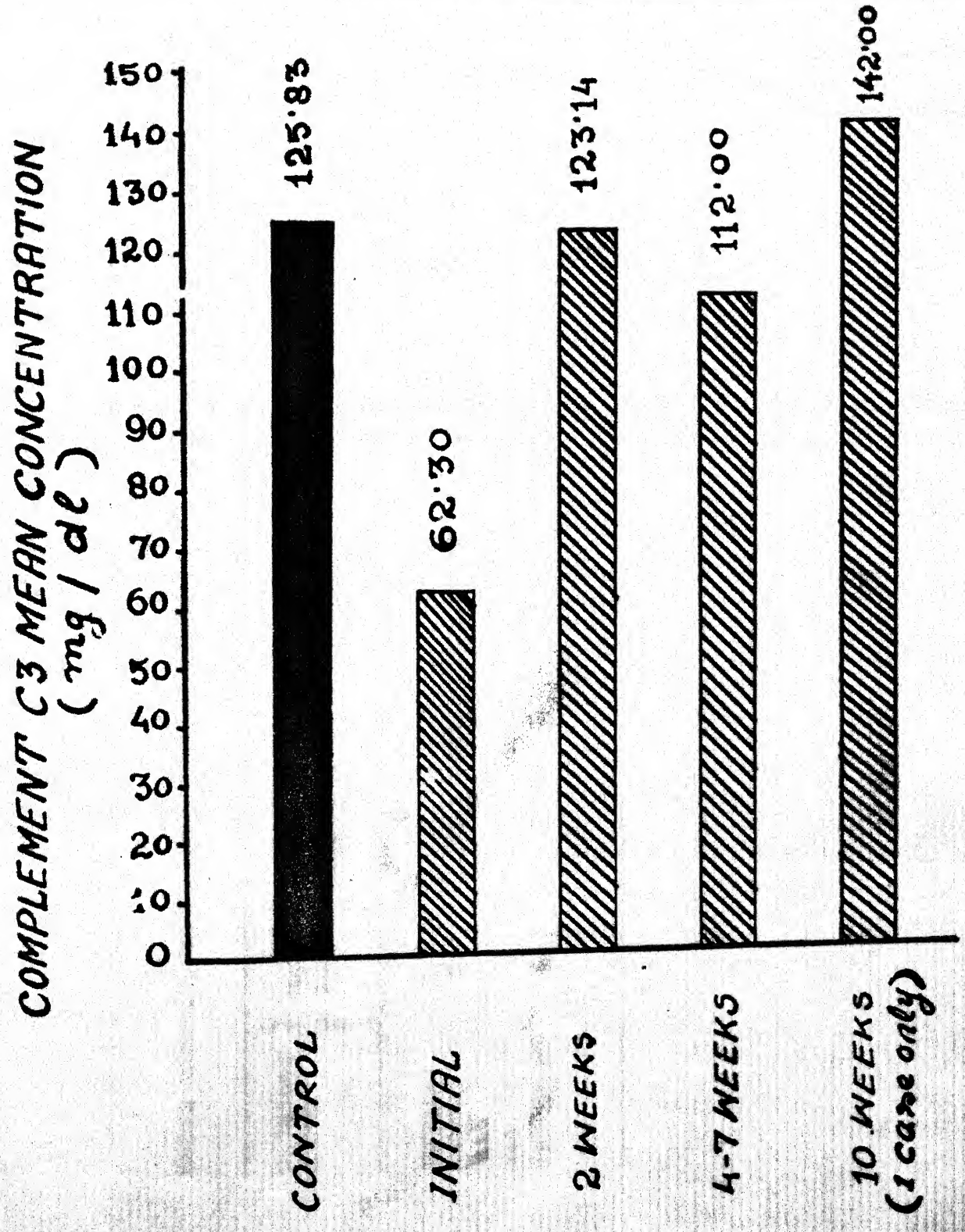

FIG. No. 10.

Table XI

Serum albumin, haemoglobin, CH_{50}, AP_{50} and C3 levels in follow up group of kuchinker.

Clinical group	No. of cases	Serum albumin (gm/dl) Mean ± S D	Haemoglobin (gm/dl) Mean ± S D	CH_{50} (U/ml) Mean ± S D	AP_{50} (U/ml) Mean ± S D	C3 (ug/dl) Mean ± S D
Initial sample	8	2.08 ± 1.62	5.60 ± 0.88	5.46 ± 3.96	31.40 ± 3.90	38.67 ± 3.31
1st follow up	1	3.90	7.00	12.86	45.00	92.00

Haemoglobin :

As with serum albumin, there was a rise of 2.00 gm/dl in haemoglobin value after 2 weeks follow up.

CH_{50} :

CH_{50} value increased by 2.58 U/ml after 2 weeks.

AP_{50} :

In contrast to other parameters, AP_{50} value decreased from 47.12 U/ml to 45.00 U/ml after a 2 weeks follow up.

Complement C3 :

On evaluation of C3 concentration, a significant rise of 52.00 ug/dl was observed in 2 weeks time (Fig.11).

Correlation of Age with Complement Activity :

Table XII shows the mean values of CH_{50}, AP_{50} and C3 complement in different age groups.

Table XII

Correlation of age with complement activity.

1 Age group (months)	No.of cases	2 CH_{50} (U/ml) Mean ± S D	3 AP_{50} (U/ml) Mean ± S D	4 C3 (mg/dl) Mean ± S D
12 - 24	22	5.68 ± 3.11	52.31 ± 22.68	81.13 ± 37.81
24 - 36	13	6.40 ± 3.38	52.99 ± 20.38	71.85 ± 40.62
36 - 48	5	5.25 ± 2.82	54.29 ± 10.71	71.80 ± 42.00
48 - 60	3	8.46 ± 3.29	58.68 ± 13.89	96.67 ± 18.04

$r_{1,2}$ = 0.162 ($P > 0.05$)
$r_{1,3}$ = -0.118 ($P > 0.05$)
$r_{1,4}$ = -0.002 ($P > 0.05$)

COMPLEMENT C3 CONCENTRATION IN INITIAL AND FOLLOWUP GROUPS OF KWASHIORKOR

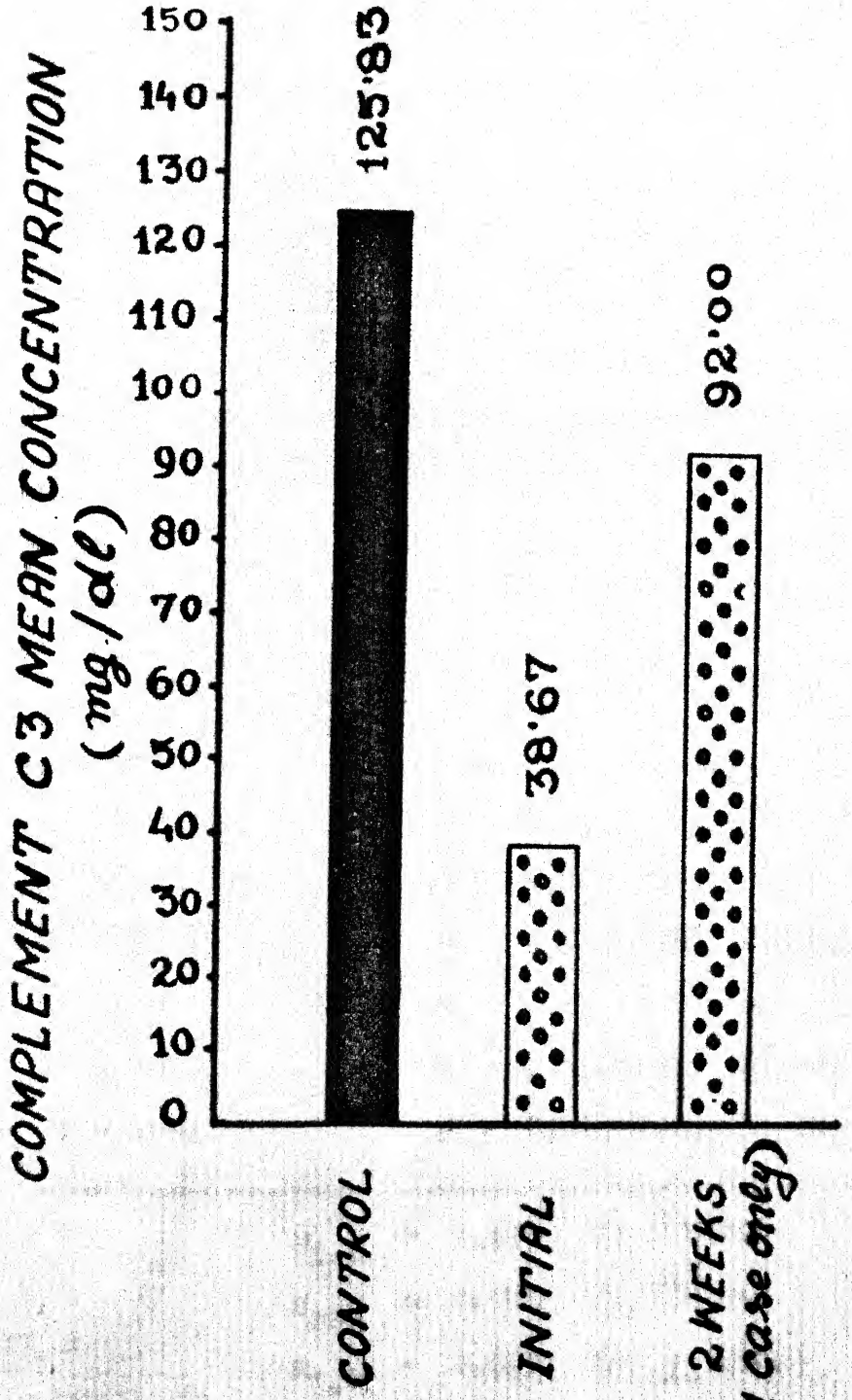

FIG. No. 11.

It is obvious from Table that no definite trend of rise or decline was seen in complement values in relation to age. Correlation coefficient (r) of age with CH_{50}, AP_{50} and C3 were observed to be 0.162, -0.118 and -0.002 respectively, all being non-significant ($P > 0.05$).

Correlation of Weight (expressed as % of 50th Percentile of Harvard Standard) with Complement Activity :

Table XIII shows the mean values of CH_{50}, AP_{50} and C3 complement in different percentage weight groups.

Table XIII

Correlation of weight (expressed as % of 50th percentile of Harvard Standard) with complement activity.

1 Weight expressed as % of 50th percentile of Harvard Standard	No.of cases	2 CH_{50} (U/ml) Mean ± S D	3 AP_{50} (U/ml) Mean ± S D	4 C3 (mg/dl) Mean ± S D
< 50%	18	4.97 ± 3.15	87.44 ± 30.43	66.15 ± 26.91
51 - 60 %	14	6.68 ± 2.98	61.76 ± 24.98	85.36 ± 21.37
61 - 70 %	8	3.98 ± 3.16	87.87 ± 22.06	68.38 ± 24.08
71 - 80 %	2	4.89 ± 0.19	93.01 ± 6.60	80.00 ± 8.49
81 - 90 %	5	7.68 ± 1.70	68.48 ± 9.02	126.20 ± 30.61
91 - 100%	7	6.78 ± 3.11	62.08 ± 10.64	126.29 ± 20.70

$r_{1,2} = 0.152$ ($P > 0.05$)

$r_{1,3} = 0.099$ ($P > 0.05$)

$r_{1,4} = 0.665$ ($P < 0.001$)

It is clear from Table that no definite trend of rise or decline was seen in CH_{50} and AP_{50} values in relation to weight percentage. Correlation coefficient (r) of weight percentage with CH_{50} and AP_{50} were observed to be 0.192 and 0.099 respectively, being non-significant ($P > 0.05$). However, a positive correlation (r = 0.685) was seen between weight percentage and C3 values, being statistically significant ($P < 0.001$).

Correlation of Length/Height (expressed as % of 50th percentile of Harvard Standard) with Complement Activity :

Mean values of CH_{50}, AP_{50} and complement C3 in different groups of length (height) percentage are shown in Table XIV.

Table XIV

Correlation of length/height (expressed as % 50th percentile of Harvard Standard) with Complement activity.

1 Length/height expressed as % of 50th percentile of Harvard Standard.	No. of cases	2 CH_{50} (U/ml) Mean ± S D	3 AP_{50} (U/ml) Mean ± S D	4 C3 (mg/dl) Mean ± S D
70 - 80 %	7	6.36 ± 3.95	53.63 ± 12.09	68.57 ± 24.36
81 - 90 %	21	5.47 ± 2.69	58.11 ± 10.14	89.05 ± 24.17
91 - 100%	16	6.50 ± 3.46	72.84 ± 22.60	109.38 ± 37.07

$r_{1,2} = 0.076$ ($P > 0.05$)
$r_{1,3} = 0.222$ ($P > 0.05$)
$r_{1,4} = 0.636$ ($P < 0.001$)

It is evident from Table that correlation coefficient (r) of length percentage with CH_{50} was observed to be 0.076, being non-significant ($P > 0.05$). Although there was

a rising trend in AP_{50} values in relation to length percentage, correlation coefficient (r = 0.222) between them was non-significant (P>0.05). However, a positive correlation (r = 0.636) was observed length percentage and C3 values, being statistically significant (P<0.001).

Correlation of Mid-arm Circumference with Complement Activity :

The mean values of CH_{50}, AP_{50} and complement C3 in different groups of mid-arm circumference are depicted in Table XV.

Table XV

Correlation of mid-arm circumference with complement activity.

1 Mid-arm circumference (cm)	No. of cases	2 CH_{50} (U/ml) Mean ± S D	3 AP_{50} (U/ml) Mean ± S D	4 C3 (mg/dl) Mean ± S D
6 - 9	10	4.77 ± 2.78	60.85 ± 24.12	87.70 ± 20.87
9 - 12	20	5.98 ± 3.87	61.96 ± 24.13	84.50 ± 24.94
12 - 15	4	7.86 ± 1.84	67.08 ± 9.12	90.50 ± 40.11
15 - 18	10	6.82 ± 1.98	87.67 ± 20.28	120.00 ± 22.87

$r_{1,2}$ = 0.265 (P>0.05)
$r_{1,3}$ = 0.142 (P>0.05)
$r_{1,4}$ = 0.721 (P<0.001)

It is obvious from the Table that no definite trend of rise or decline was seen in CH_{50} and AP_{50} values in relation of mid-arm circumference. Correlation coefficients (r) of mid-arm circumference with CH_{50} and AP_{50} were found to be 0.265 and 0.142 respectively, being non-significant (P>0.05). However, there was definite rising

trend in C3 values in relation to arm circumference, correlation coefficient (r) being 0.721 and significant ($P < 0.001$).

Correlation of Serum Albumin Concentration and Complement Activity :

Table XVI shows the mean values of CH_{50}, AP_{50} and complement C3 in different groups of serum albumin values.

Table XVI

Correlation of serum albumin concentration with complement activity.

1 Serum albumin (gm/dl)	No. of cases	2 CH_{50} (U/ml) Mean ± S D	3 AP_{50} (U/ml) Mean ± S D	4 C3 (mg/dl) Mean ± S D
< 2	2	3.24 ± 1.08	33.34 ± 1.73	38.00 ± 2.83
2 - 3	6	5.08 ± 2.44	53.26 ± 11.20	84.67 ± 19.09
3 - 4	24	6.22 ± 2.72	66.65 ± 24.98	73.04 ± 29.32
4 - 5	12	6.96 ± 2.25	89.88 ± 12.88	107.67 ± 27.21

$r_{1,2}$ = 0.899 ($P < 0.05$)
$r_{1,3}$ = 0.107 ($P > 0.05$)
$r_{1,4}$ = 0.828 ($P < 0.001$)
$r_{2,3}$ = -0.222 ($P > 0.05$)
$r_{2,4}$ = 0.548 ($P < 0.05$)
$r_{3,4}$ = -0.029 ($P > 0.05$)

Table depicts that definite trend of rise was seen in CH_{50} and C3 values in relation to serum albumin values. Correlation coefficients (r) of serum albumin with CH_{50} and C3 were observed to be 0.899 and 0.828 respectively, being

significant ($P < 0.05$ and < 0.001 respectively). However, a non-significant ($P > 0.05$) correlation ($r = 0.107$) was found between serum albumin and AP_{50} values.

On further evaluation, it was observed that there was a positive correlation ($r = 0.348$) of C3 with CH_{50} values, being statistically significant ($P < 0.05$). On comparison of C3 values with AP_{50} values, correlation coefficient (r) was found to be -0.089, being non-significant ($P > 0.05$). Similarly correlation between CH_{50} and AP_{50} ($r = -0.222$) was found to be non-significant ($P > 0.05$).

Correlation of Haemoglobin Values with Complement Activity :

Mean values of CH_{50}, AP_{50} and complement C3 in different groups of haemoglobin values are shown in Table XVII.

Table XVII

Correlation of haemoglobin values with complement activity.

1 Haemoglobin (gm/dl)	No. of cases	2 CH_{50} (U/ml) Mean ± S D	3 AP_{50} (U/ml) Mean ± S.D	4 C3 (mg/dl) mean ± S D
5 - 8	16	6.06 ± 3.44	54.69 ± 11.96	63.81 ± 32.04
8 - 11	17	8.00 ± 3.42	68.06 ± 28.48	61.76 ± 29.98
11 - 14	11	7.33 ± 2.00	63.76 ± 10.04	126.91 ± 24.04

$r_{1,2} = 0.114$ ($P > 0.05$)
$r_{1,3} = 0.196$ ($P > 0.05$)
$r_{1,4} = 0.628$ ($P < 0.001$)

It is clear from Table that there was no definite rising or declining trend in CH_{50} and AP_{50} values in relation

to haemoglobin values. Correlation coefficients(r) of haemoglobin values with CH_{50} and AP_{50} values were noticed to be 0.114 and 0.196 respectively, being non-significant (P $\overline{>}$0.05). On comparison of haemoglobin values with CH values, a positive correlation coefficient (r = 0.638) was observed, being statistically significant (P $\lesssim$0.001).

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DISCUSSION

The present work has been carried to study the complement activity in 32 pre-school children (1-5 years age) suffering from protein-calorie malnutrition and 12 nutritionally normal age matched children, serving as control. The study was conducted at M.L.B. Medical College, Jhansi between May 1981 and March 1982.

The primary aim of our study was to evaluate the complement profile in children suffering from PCM and compare the values with those obtained in control cases. Besides evaluating the complement activity traditional parameters viz. weight, length/height and mid-arm circumference, serum albumin and blood haemoglobin were used to assess the nutritional status of children. Complement activity was assessed by total haemolytic complement (CH_{50}) activity, alternative pathway activity (AP_{50}) and C3 concentration. It was also our endeavour to ascertain the possible interrelationship between the clinical progress, following nutritional rehabilitation and the subsequent change in complement activity. With the objective in view, complement activity, anthropometric measurements, serum albumin and blood haemoglobin values were evaluated at the time of initial contact and in subsequent follow ups at 2 weeks, 4-7 weeks and 10-12 weeks interval. Statistical analysis was done to derive means and standard deviations (SD). An attempt was made to correlate age, weight, length/height,

mid-arm circumference, serum albumin and blood haemoglobin with various parameters of the complement activity.

The PCM group in our study was further classified into 19 cases of marasmus, 12 cases of marasmic-kwashiorkor and 9 cases of kwashiorkor according to Wellcome classification. All the cases belonged to low socioeconomic status and most of them were suffering from gastrointestinal and respiratory tract infections. Care was however taken to exclude the cases in whom secondary factors thought to affect complement activity could have been operational. History of past illness and family history were noted in each case. All the children at the initial contact were receiving diet, grossly deficient in calories and proteins. None of the twelve control cases was suffering from any demonstrable illness at the time of inclusion in this study.

Based on observations depicted in tables I to XVII, various inferences have been drawn and discussed under different headings.

ANTHROPOMETRIC PROFILE :

Anthropometric profile of both the control and PCM cases at the time of initial contact (Table III) revealed that the mean weight, length (height) and mid-arm circumference in all 3 groups of PCM viz. marasmus, marasmic-kwashiorkor and kwashiorkor were appreciably less than those in healthy controls. However, groups of PCM among themselves did not show clear differences in anthropometric values. Subsequently,

during follow up, after nutritional rehabilitation and subsidence of infection, it was seen that mean weight and mid-arm circumference increased consistently during 1st, 2nd and 3rd follow ups in cases of marasmus and marasmic-kwashiorkor and during the 1st follow up in a single case of kwashiorkor.

It was also observed that the sustained improvement in weight and mid-arm circumference was more marked in cases of marasmus. However, there was no improvement seen in mean length (height) at subsequent follow ups in the 3 groups of PCM cases, exception being a single case of kwashiorkor. These findings are not surprising since it is known that body weight is a more sensitive indicator of nutrition and that evaluation of linear body change is usually possible at longer intervals of time.

Correlation of Weight (expressed as % of 50th percentile of Harvard Standard) with Complement Activity :

From Table XXII it is obvious that no definite trend of rise or decline was seen in CH_{50} values in relation to change in percentage weight. Correlation coefficients of percentage weight with CH_{50} and AP_{50} were 0.192 and 0.099 respectively, being non-significant. However, a positive significant correlation (r = 0.665) was seen between the percentage weight and C3 values.

Correlation of Length/Height (expressed as % of 50th percentile of Harvard Standard) with Complement Activity :

Correlation coefficient of length (height) percentage

with both CH_{50} and AP_{50} values was non-significant, while C3 values (r = 0.636) was statistically significant, as shown in Table XIV.

Correlation of Mid-Arm Circumference with Complement Activity:

The correlation of the mid-arm circumference with complement activity (Table XV) revealed that there was no definite rise or decline in CH_{50} and AP_{50} values with the change in mid-arm circumference, correlation coefficients(r) being 0.265 and 0.142 respectively. As with weight and length/height, it was observed that there was a definite rising trend in C3 values in relation to arm circumference, correlation coefficient being 0.721.

Thus we see that the percentage of weight, height (length) and mid-arm circumference had a positive and significant correlation with complement C3 levels, while CH_{50} and AP_{50} values had no significant correlation with anthropometric measurements. Our observations are comparable with the findings of other workers in the field. Ghai et al (1976) in their study to evaluate the complement profile in PCM cases, noted that with refeeding, C3 was the first complement component to show a significant rise, followed by C9 and C6, thereby concluding that out of all the complement components, C3 was the most sensitive index of nutritional status. Kielmann et al (1976), in their study of complement profile in pre-school children has also shown a positive correlation of weight, length (height) and arm circumference with C3 values. Thus the findings of the present study and that of other workers clearly reveal that weight and length (height)

percentage, and arm circumference show a positive correlation with only one parameter of complement activity viz. C3 levels, suggesting that with nutritional rehabilitation, there is not only an improvement in the growth and development, but also an improvement in the immunological status of the child.

SERUM ALBUMIN AND HAEMOGLOBIN VALUES :

It is obvious from Table IV that the mean serum albumin and haemoglobin values were significantly depressed in PCM cases as compared to the controls ($P < 0.001$). Similar findings have been observed by other workers in the field (Gluzi et al, 1976 and Haller et al, 1978). Further it was observed that mean serum albumin and haemoglobin values were lowest in cases suffering from kwashiorkor (Table V and Fig. 5) at the initial contact. After nutritional repair there was a sustained rise in mean serum albumin levels, during the 3 follow ups, in marasmus, marasmic-kwashiorkor cases. Similarly rise in serum albumin was seen during the 1st follow up in a single case of kwashiorkor (Table IX, X and XI). Like serum albumin level, mean haemoglobin level also increased substantially in the all 3 follow ups in marasmus and marasmic-kwashiorkor and during the 1st follow up in single case of kwashiorkor. Thus it was apparent that nutritional repair, with adequate amount of calories and proteins, led to a sustained rise of serum albumin and haemoglobin values in all groups of PCM. Evaluation of these two parameters of nutrition and their

correlation with the complement activity has not hitherto been attempted by other workers and hence comparison to other studies could not be done.

COMPLEMENT PROFILE IN STUDY GROUPS :

I CONTROLS :

Total Haemolytic Complement (CH_{50}) Activity :

In the present study, total haemolytic complement (CH_{50}) had mean values of 7.16 ± 1.98 U/ml. It was seen that values from our study were much lower than those obtained by other workers. Smythe et al (1971) found CH_{50} values in the range of 1/128 - 1/512. Chandra (1972) and Jagadeesan and Reddy (1979) reported mean values of 58.00 ± 12.00 and 66.10 ± 3.21 U/ml respectively. However, Sushini et al (1976) found very high values of CH_{50} viz. 380.00 ± 80.00 U/ml in their study. The possible explanation for vide variation in CH_{50} values, obtained by various authors, could be that estimation of CH_{50} depends upon the standardization techniques used in different laboratories.

Since all control cases in our study were free of infections, we could not predict the effect of infection on the value of CH_{50} in well-nourished children.

Alternative Pathway Activity (AP_{50}) :

In our study mean value of AP_{50} were found to be 64.70 ± 10.11 U/ml. Inspite of our best efforts reference values of AP_{50} (a measure of alternative pathway activity) could not be found in literature for comparative study.

Complement C3 Values :

C3 complement values (mg/dl), considered to be most sensitive index of complement activity, had mean values of 125.63 ± 23.90 in normal control children. These values were found to be consistent with those obtained by various other workers viz. Chandra (1973)[6], Sirisinha et al (1973)[7], Chandra (1975)[8], Otani et al (1976)[9], Kielmann et al (1976)[10], Keller et al (1978)[11] and Jagadeesan and Reddy (1979)[12]. However Neuman et al (1975) found slightly lower values of C3 i.e. 86.90 ± 2.40 mg/dl.

Chandra (1975), in his study to evaluate the serum complement levels in malnutrition and well-nourished children, observed that the complement C3 value was much higher (245.00 ± 57.00 mg/dl) in well-nourished children with infection than the other group of well-nourished children without infection (132.00 ± 18.00 mg/dl). The author however could not ascribe any explanation of the hightened C3 levels with infection in well-nourished children. Since all the 12 control children in our study were free of infection, we could not arrive at a definite conclusion, regarding the effects of infections on C3 complement levels.

II PROTEIN-CALORIE MALNUTRITION :

INITIAL CONTACT :

Total Haemolytic Complement (CH_{50}) Activity :

It is evident from Table IV that at the time of initial contact, mean CH_{50} value in the PCM group was lower

than the value in control group, the difference between the two values was statistically non-significant ($P > 0.05$).

On further analysis it was observed that though the values of CH_{50} in marasmus, marasmic-kwashiorkor and kwashiorkor were lower than the controls (Table V and Fig. 6), these values were not significantly different from the controls ($P > 0.05$). Similarly the group differences of mean CH_{50} values were not appreciably different from each other. In contrast to our study, Smythe et al (1971) and Chandra (1975) reported significantly lowered values of CH_{50} in infants and children with PCM. Sunkind et al (1976), in an elaborate study to assess the complement activity in 36 children with severe PCM, using the CH_{50} titre as the parameter, reported a significant depression of CH_{50} levels, only in cases of kwashiorkor, as compared to the controls. However, on comparing the CH_{50} values of children with marasmus and marasmic-kwashiorkor to those of controls, they did not observe any significant difference. Keller et al (1978) and Jagadeesan and Reddy (1979) have also observed a more marked depression of CH_{50} values in kwashiorkor than in marasmus and marasmic-kwashiorkor cases.

The results of the present study therefore reveal, that, though CH_{50} values were lower in all groups of PCM, a statistically significant difference as compared to controls, could not be elucidated. Further, unlike other workers, we could not arrive at any correlation between the CH_{50} values and the severity of PCM; values in all 3 groups remained

were or less equal. This was in contrast to the results obtained by other workers, where not only CH_{50} values in PCM were statistically different from the controls but maximum depression was also observed in the most severe form of PCM viz. kwashiorkor.

Alternative Pathway Activity (AP_{50}) :

It is evident from Table IV that the mean AP_{50} values in the PCM group was not statistically different from the controls. Further the mean AP_{50} values in marasmus, marasmic-kwashiorkor and kwashiorkor were found to 63.82 ± 24.91, 62.33 ± 22.83 and 61.40 ± 3.90 respectively (Fig.:7). Although these values in 3 groups of PCM were lower, only the value in kwashiorkor group was significantly lower ($P < 0.05$) as compared to the controls. This suggests that the alternate pathway activity was adversely affected only in the kwashiorkor group, values being unaffected in marasmus and marasmic-kwashiorkor cases. Since there is paucity of data regarding AP_{50} in literature, a comparison of these values could not be ascertained.

However, Sirisinha et al (1973) and Muller et al (1978) studied the alternative pathway activity by evaluating the concentration of factor B (named C3 pro-activator, previously) and observed that this factor was depressed in children suffering from PCM. Since these authors found normal C4 levels, which are depressed in classical pathway activity, they suggested that in PCM, alternative pathway was also activated. However, in the present study, alternative pathway activity was found to be unaffected except in kwashiorkor. The possible mechanism of depressed AP_{50} (alternative pathway activity) values could be that

infection triggered alternative pathway thereby resulting in diminished concentration of AP_{50} values. Since in our study, all the cases of kwashiorkor were severely infected, maximum depression of AP_{50} values was obtained in this group. Further, a significant reduction in alternative pathway activity could have accounted for lowering the antimicrobial resistance therefore resulting in severe infection in kwashiorkor.

Complement C3 Values :

The complement C3 levels in PCM group had mean value of 60.59 ± 23.25 mg/dl. These values were found to be statistically lower than those in controls (Table IV). On further analysis of the C3 levels in marasmus, marasmic-kwashiorkor and kwashiorkor it was observed that the values in all 3 groups were significantly lower as compared to controls ($P < 0.001$), maximum depression being observed in cases of kwashiorkor. However, the mean values in the 3 groups of PCM were not appreciably different from each other (Table V and Fig. 8). A significant depression of C3 level in PCM cases was also obtained by various other workers in the field viz. Chandra (1972), Sirisinha et al (1973), Chandra (1975), Neuman et al (1975), Ohmi et al (1976), Kielmann et al (1976), Muller et al (1978), Kielmann and Cursio (1979) and Jagadeesan and Reddy (1979).

It was further observed in the present study that a definite correlation existed between the serum albumin concentration and complement C3 levels ($r = 0.625$). In the

kwashiorkor group concentration of serum albumin was the lowest and maximum depression of the C3 level was also obtained in these cases (Table V), thus suggesting a direct correlation of serum C3 values with the severity of malnutrition.

Correlation of Age with Complement Activity :

No definite trend of rise or decline was seen in complement values viz. CH_{50}, AP_{50} and C3 in relation to age, thus suggesting no effect of age on complement activity. This aspect has not been evaluated so far by any other worker.

Correlation of Serum Albumin Values with Complement Activity :

It is evident from Table XVI, that when serum albumin levels were correlated with the CH_{50}, AP_{50} and C3 levels, a definite and positive rise was observed only in CH_{50} and C3 levels with the increasing levels of albumin. Correlation coefficients (r) of serum albumin with CH_{50} and C3 were 0.299 and 0.625 being significant at $P < 0.05$ and < 0.001 respectively. However, no rise in AP_{50} values was observed with the increasing levels of serum albumin ($r = 0.107$).

On further evaluation, it was observed that there was a positive correlation ($r = 0.346$) of C3 with CH_{50} values, being statistically significant ($P < 0.05$). However on comparison of C3 and CH_{50} values with AP_{50} values, correlation coefficients (r) were found to be -0.039 and -0.222 respectively, both being non-significant (> 0.05).

A positive significant correlation between C3 and CH_{50} values implies that a change in the concentration of C3 will reflect on CH_{50} values. In this regard Spitzer(1977b) reported that CH_{50} values were affected only when there was approximately 80% decrease in C3 levels. However, since negative non-significant correlation was observed between C3 and AP_{50} values, the values of these two parameters would thus be independent of each other in PCM cases.

A similar significant correlation between serum albumin levels and complement activity was also observed by Sirisinha et al (1973), Haller et al (1978) and Jagadeesan and Reddy (1979). They reported that there was a direct correlation between the degree of complement depletion (especially C3 levels) to the severity of the depletion of various plasma proteins.

Sirisinha et al (1973), Haller et al (1978) and Jagadeesan and Reddy (1979) thus suggested that decreased protein synthesis in the liver played a major role in the reduction of C3 levels and hence in the impairment of complement system in PCM. However, Kielmann and Cureio (1979) showed that there was no significant correlation between total serum proteins and C3 levels.

Correlation of Haemoglobin Values with Complement Activity :

An attempt was made to correlate the haemoglobin values with the complement activity (Table XVII). We observed that there was no definite rising or decling trend

in CH_{50} and AP_{50} values in relation to haemoglobin values, correlation coefficients (r) being 0.114 and 0.196 respectively ($P > 0.05$). However, a significant positive correlation ($r = 0.628$) was observed between haemoglobin and C3 values.

A significant correlation between haemoglobin and C3 values in our study suggest that haemoglobin per se, could also be one of the factors responsible for depression of C3 levels in PCM cases. However, a study conducted by Kielmann and Cureio (1979) did not show any significant correlation of C3 levels with haemoglobin values.

Complement Activity and Infection :

In the present study since all the PCM cases were suffering from infection, it was not possible to elucidate whether there was any subsequent difference in the complement activity between infected and non-infected cases. Sirisinha et al (1973), Chandra (1978), Soukind et al (1976) and Kielmann and Cureio (1979) have all reported that there was pronounced depression of complement activity (especially CH_{50} and C3 levels) in PCM children suffering from infection than in those without it. Further they also reported difference in the complement levels with severity of infection, levels being lower in severely infected cases than in those having mild infection.

FOLLOW UP :

After the initial assessment of anthropometry, serum albumin, haemoglobin and complement activity in the PCM cases, all of them were put on nutritional rehabilitation schedule and infections were treated by appropriate antibiotics. An attempt was made to follow all PCM cases with repeat anthropometry, estimation of serum albumin, haemoglobin and complement activity.

Total Haemolytic Complement (CH_{50}) Activity :

It is evident from Tables IX, X and XI that after nutritional repair, CH_{50} levels attained maximum mean values after the 1st follow up in all the cases of marasmus and marasmic-kwashiorkor cases and also in a single case of kwashiorkor. Although in the 2nd and 3rd follow up, mean levels had a declining trend in both marasmus and marasmic-kwashiorkor cases, yet the levels remained higher than that obtained at initial contact. Since the solitary case of kwashiorkor left the hospital after 1st follow up, subsequent CH_{50} level could not be ascertained. Our results are consistent with the findings of Chandra (1975), Suskind et al(1976) and Jagadeesan and Reddy (1979).

Alternative Pathway Activity (AP_{50}) :

Tables IX, X and XI clearly demonstrate that on evaluation of AP_{50} values at follow up in different groups of PCM, no consistent pattern of rise was observed. Since there is a paucity of available literature on AP_{50} values, comparison of our values could not be elucidated.

Complement C3 Values :

It is evident from Table IX, X and XI, and Fig.9, 10 and 11 that after nutritional rehabilitation C3 values, attained maximum mean level after the 1st follow up, in only marasmic-kwashiorkor. Values in marasmus showed a definite increasing trend till the 2nd follow up. In a single case of kwashiorkor followed only once, significant rise of C3 level was observed. Rise of C3 levels after nutritional rehabilitation was also observed by Sirisinha et al (1973), Chandra (1975), Giusi et al (1976) and Jagadeesan and Reddy (1979).

A critical analysis of the complement activity on follow up in the 3 groups of PCM revealed that the levels of most complement components, after nutritional rehabilitation, rose to above control values. The mechanism involved in such overshoot or rebound is still not known. However, Sirisinha et al (1973) are of the view that after complement depletion in PCM, there is an accentuated synthesis of the complement proteins and a state of over production ensues, accounting for above the normal values of complement components.

Thus the findings on the complement activity in children suffering from protein-calorie malnutrition, in the present study, reveal certain interesting observations. That the complement profile was significantly depressed in PCM cases as compared to the controls. It was seen that the

depression of complement activity was mainly accounted by a significant depression of the complement C3 level, while CH_{50} and AP_{50} values, though lower were not statistically significant from the controls. Another significant finding of our study was highly significant depression of complement C3 ($P < 0.001$) in all 3 groups of PCM, levels being maximally depressed in kwashiorkor. However, though CH_{50} values were lower in all the groups, no statistical significance was observed as compared to the controls ($P > 0.05$). AP_{50} values, on the other hand, were found to be depressed only in kwashiorkor ($P < 0.05$), while no significant difference was obtained in marasmus and marasmic-kwashiorkor. Since C3 levels were found to be depressed in all forms of malnutrition, it was inferred that probably complement C3 was the most sensitive index of nutritional status. The findings, that CH_{50} levels were more or less equal in all the groups of PCM and also did not show any significant difference from the controls, demonstrated that this parameter was not a very good index of evaluation of complement activity, a change in CH_{50} levels could possibly be affected by at least a 50% reduction in C3 levels (Spitzer, 1977b).

In the present series an attempt was made to evaluate the various factors which could possibly have an adverse influence on the complement profile. On the basis of a positive correlation of nutritional status (measured by

anthropometric indices), serum albumin and haemoglobin values with the complement activity, we arrived at the following possible explanations for the depression of complement system :

1. Reduction in Protein Synthesis :

This was the single most important factor as observed in present study to cause depression of complement activity. This finding is substantiated by the following observations :

(a) There was a uniform depression of serum albumin levels in all the groups of PCM. Further it was observed that greater the reduction of albumin levels, more significant was the depression of complement activity, the correlation coefficient between serum albumin and C3 levels being highly significant ($P < 0.001$).

(b) With nutritional repair, there was not only a substantial increase in serum albumin levels but also concomitant rise in complement activity.

These observations in our study suggested that nutritional status of the individual could influence the complement system.

2. Reduction in Haemoglobin Values :

Since a definite correlation was observed between haemoglobin levels and complement C3 activity, one could suggest that haemoglobin levels played an important role in the depression of complement levels.

Various hypothesis have been put forward by different workers to explain the possible mechanisms of depression of complement activity in PCM. Sirisinha et al (1973), Chandra (1975) and Suskind et al (1976) are unanimous in their opinion that the child's nutritional status was probably the major factor in causing the depression of complement activity. Chandra (1975) further stated that since liver damage occurred in PCM and liver was the main site of C3 synthesis, reduction of C3 levels could be a corrollary to liver damage. Another important factor to cause depression of complement activity could be a phenomenon of complement consumption which occurred in the presence of infection. Suskind et al (1976) suggested that anticomplementary activity in serum of PCM cases could also contribute to depression of CH_{50}. Other possible explanations for depression of complement system in malnourished children, though unimportant, could be the changes in blood vascular compartment occuring in malnutrition (Chandra, 1975). Complement depletion could also occur as a result of protein-losing gastroenteropathy in PCM cases (Chandra, 1975).

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SUMMARY & CONCLUSION

The present study on complement system in children with protein-calorie malnutrition was conducted in the Department of Paediatrics, M.L.B. Medical College, Jhansi (U.P.). Thirty two pre-school children (1-5 years age) suffering from protein-calorie malnutrition and twelve age matched well-nourished healthy controls comprised the material for the present study. Complement activity was assessed by three parameters viz. total hemolytic complement (CH_{50}) activity, alternative pathway activity (AP_{50}) and serum complement C3 levels. Besides complement activity, various anthropometric measurements (weight, length/height and mid-arm circumference), serum albumin and blood haemoglobin values were noted in each case.

Children suffering from PCM were treated and put on nutritional rehabilation schedule. An attempt was made to follow the cases at 2 weeks, 4-7 weeks and 10-12 weeks interval.

For the purpose of analysis children suffering from PCM were divided into 3 groups viz. marasmus, marasmic-kwashiorkor and kwashiorkor according to Wellcome classification.

From the data collected means, standard deviations and correlation coefficients were calculated. Means of PCM cases were compared and discussed in relation to controls.

ANTHROPOMETRIC PROFILE :

Anthropometric values viz. weight, length/height and mid-arm circumference in children suffering from PCM were appreciably less than in controls. However, different groups of PCM as per Wolaroi classification, did not reveal any clear differences.

Following nutritional rehabilitation, mean weight and mid-arm circumference increased consistently during 1st, 2nd and 3rd follow ups in cases of marasmus and marasmic-kwashiorkor and during the 1st follow up in a single case of kwashiorkor. However, there was no improvement seen in mean length (height) at subsequent follow ups in all the 3 groups of PCM, exception being a single case of kwashiorkor.

SERUM ALBUMIN VALUES :

Mean serum albumin level (3.13 ± 0.80 gm/dl) was found to be significantly depressed in PCM as compared to the controls (4.06 ± 0.37 gm/dl).

Mean serum albumin levels (gm/dl) in marasmus, marasmic-kwashiorkor and kwashiorkor were 3.49 ± 0.89, 2.72 ± 0.41 and 2.00 ± 1.62. These values were significantly lower than those of controls. Value was lowest in cases suffering from kwashiorkor.

Following nutritional rehabilitation there was increase in mean serum albumin levels in all 3 PCM groups.

HAEMOGLOBIN VALUES :

Mean haemoglobin level was significantly lower in PCM cases (7.83 ± 1.62 gm/dl) as compared to controls (12.87 ± 1.04 gm/dl).

Mean haemoglobin values (gm/dl) i.e. 8.62 ± 1.14, 6.96 ± 1.60 and 5.60 ± 0.83 in marasmus, marasmic-kwashiorkor and kwashiorkor, being lowest in last group, were also significantly lower as compared to controls.

During follow up, as with serum albumin levels, there was a definite rise in mean haemoglobin value in each of the 3 groups of PCM.

COMPLEMENT ACTIVITY :

Total Haemolytic Complement (CH_{50}) Activity :

Mean CH_{50} value (U/ml) in PCM cases (5.89 ± 3.45) was not significantly different from controls (7.16 ± 1.92).

Mean CH_{50} values (U/ml) in marasmus, marasmic-kwashiorkor and kwashiorkor were found to be 5.81 ± 3.88, 5.77 ± 2.70 and 5.48 ± 3.96 respectively. These values were not significantly different from the controls. Also, the group differences of mean CH_{50} values were not appreciably different.

During follow up there was no constant pattern of rise or decline in mean CH_{50} values in marasmus and marasmic-kwashiorkor cases. However, in single case of kwashiorkor followed only once, there was an increase in CH_{50} value by 2.88 U/ml.

Alternative Pathway Activity (AP_{50}) :

Mean AP_{50} value (U/ml) in PCM cases (61.86 ± 22.07) was not significantly different from controls (64.70 ± 10.11).

Mean AP_{50} values (U/ml) in 3 group of PCM viz. marasmus, marasmic-kwashiorkor and kwashiorkor were found to be 63.82 ± 24.91, 62.22 ± 22.53 and 51.40 ± 3.90 respectively. Out of all these values, mean value in kwashiorkor group was significantly lower as compared to controls. However, there were no significant differences of AP_{50} means within the PCM groups.

During follow up periods, there was no constant difference in repeat AP_{50} values in marasmus and marasmic-kwashiorkor. However, in a single case of kwashiorkor value decreased from 47.12 U/ml to 45.00 U/ml after 1st follow up.

Complement C3 Values :

Mean complement C3 value in children suffering from PCM (60.89 ± 23.23 mg/dl) was significantly lower as compared to controls (125.62 ± 23.98 mg/dl).

Mean C3 values (mg/dl) in marasmus, marasmic-kwashiorkor and kwashiorkor groups were found to be 63.16 ± 23.98, 62.30 ± 22.88 and 38.67 ± 2.31 respectively. These values were significantly lower as compared to controls. However, mean values in 3 groups were not significantly different from each other.

In children suffering from marasmus mean C3 values rose consistently even above control levels, after 1st and 2nd follow up. In a single case, followed 3rd time, a rise from 88.00 mg/dl to 114 mg/dl was noticed.

During follow up in marasmic-kwashiorkor cases, though C3 values attained a significantly higher levels, there was no consistent rising pattern.

In kwashiorkor group, a rise of 52.00 ug/dl in C3 value was observed in 2 weeks time in a single case followed.

CORRELATION OF DIFFERENT PARAMETERS WITH COMPLIMENT ACTIVITY:

Age with Complement Activity :

No definite trend of rise or decline was seen in complement values viz. CH_{50}, AP_{50} and C3 in relation to age.

Height (expressed as % of 50th percentile of Harvard Standard) with Complement Activity :

No definite trend of rise or decline was seen in CH_{50} and AP_{50} values in relation to weight percentage. However, a significant positive correlation (r = 0.685) was observed between weight percentage and C3 values.

Length/Height (expressed as % of 50th percentile of Harvard Standard) with Complement Activity :

There was non-significant correlation seen between percentage length (height) and CH_{50} as well as AP_{50} values. However, a positive significant correlation (r = 0.636) was seen between length percentage and C3 values.

Mid-Arm Circumference with Complement Activity :

No definite trend of rise or decline was seen in CH_{50} and AP_{50} values in relation to mid-arm circumference. However, there was positive significant correlation (r = 0.721) between mid-arm circumference and C3 values.

Serum Albumin Values with Complement Activity :

Correlation coefficients (r) of serum albumin with CH_{50} and C3 values were 0.299 and 0.828 respectively, being significant. However, there was no significant correlation between serum albumin and AP_{50} values.

Haemoglobin Values with Complement Activity :

There was no definite rising or declining trend in CH_{50} and AP_{50} values in relation to haemoglobin values. However, there was a significant positive correlation ($r = 0.628$) between haemoglobin and C3 values.

Correlation among CH_{50}, AP_{50} and Complement C3 Values :

A positive significant correlation ($r = 0.348$) was observed between CH_{50} and C3 values. However, no significant correlation was found between CH_{50} and AP_{50} as well as between C3 and AP_{50} values.

COMPLEMENT ACTIVITY AND INFECTION :

Since all control cases were free from infection and every PCM case had one or the other type of infection, we could not predict the effect of infection on the complement activity.

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SAMPLE CASE SHEET

CASE SHEET

NAME : CASE NO.:

Date of birth : Sex: Male/Female

Age :

Father's Name :

Address :

Occupation : Father- Mother-

Total Income of Family: Rs. /Month.

Per capita income : Rs. /Month.

Birth order of child:

Geniological tree :

Dietary History:	Started at age	Upto age	Dilution
Breast Milk			
Artificial Milk only			
Added artificial milk			
Solids added			

Present diet :

Calories Proteins Adequate / inadequate

IMMUNISATION HISTORY :

	Smallpox	B.C.G.	Polio(oral)	Triple(D.P.T.)
I				
II				
III				
IV				
V				

ANTENATAL & NATAL HISTORY :

Gest. period	Birth Wt.	Drug intake	Significant illness

NOTE :

POST NATAL HISTORY (First 4 weeks):

No problem Fever Sepsis Jaundice Cyanosis Others.

MILE STONES (DEVELOPMENTAL BEHAVIOUR) :

MOTOR : AGE :

1. Head control
2. Sitting
3. Crawling
4. Standing with support
   without support
5. Walking
6. Running

Comments if any.

MANIPULATIVE :

1. Grasp.
2. Self feeding : Spoon
   Cup
3. Help in Dressing.

Comments if any.

SOCIAL :

1. Smile (Social)
2. Response to call by name
3. Sphincter control : Bladder Day Night
   Bowel Day Night

Comments if any.

SPEECH :

1. Single word (Mamma etc.)
2. Jargon
3. Small broken sentences
4. Long sentences

Comments if any.

FAMILY HISTORY :

PRESENT ILLNESS :

PAST ILLNESS :

1. Category i
2. Category ii
3. Definite H/O Primary complex
4. H/O Pertusis

   Measles

   Worm infestation

Note :- Category i - H/O acute illness viz. fever, vomiting, diarrhoea, convulsion etc. lasting more than 4 days during the previous two weeks.

Category ii- H/O cough, fever, vomiting, convulsion, diarrhoea etc. lasting more than 2 weeks any time during the previous six months.

CLINICAL EXAMINATION

GENERAL APPEARANCE :

Healthy
Malnourished

DATE

Parahemetor changes :

Fretful
Listless

HAIR :

Normal
Dyspigmentation
Easy pluckability
Sparseness

FACE :

Moon Face

EYES :

Conjunctival xerosis
Bitot's spots
Pale conjunctiva

MOUTH :

Angular stomatitis
Cheilosis
Glossitis
Swollen bleeding gums.

Dentition :

Thyroid gland :

Goitre

SKIN :

Oedema (Bilateral)
Follicular hyperkeratosis
(Type I)
Pellagrous dermatosis
Flaky paint dermatosis
Diffuse depigmentation
Measle dermatosis

Loss of Subcutaneous Fat :

Muscle Wasting :

DATE

SKELETON :

- Epiphyseal enlargement (Wrist)
- Rickety Rosary
- Persistently open ant. font.
- Harrison's sulcus
- Bossing of skull
- Knock knees
- Bow legs.

NOTE :

ABDOMEN :

- Liver
- Spleen
- Pot Belly
- Any other.

C.V.S. :

- Normal
- Any abnormality

RESPIRATORY SYSTEM :

- Normal
- Any abnormality

C.N.S. :

- Normal
- Any abnormality

ANTHROPOMETRY :

- Weight
- Length / Height
- Mid-arm circumference

NOTE :

INVESTIGATIONS

DATE

BLOOD :

- Haemoglobin
- T.L.C.
- D.L.C.
- Total Serum proteins
- Serum albumin
- Serum globulins
- AG Ratio

URINE :

STOOL :

X-ray chest
(If necessary) :

IMMUNOLOGICAL
INVESTIGATIONS :

Total Haemolytic
complement
(CH_{50}) titre :

Alternative Pathway
Activity (AP_{50}) :

Serum Complement
C3 level :

Other Relevant Investigation :